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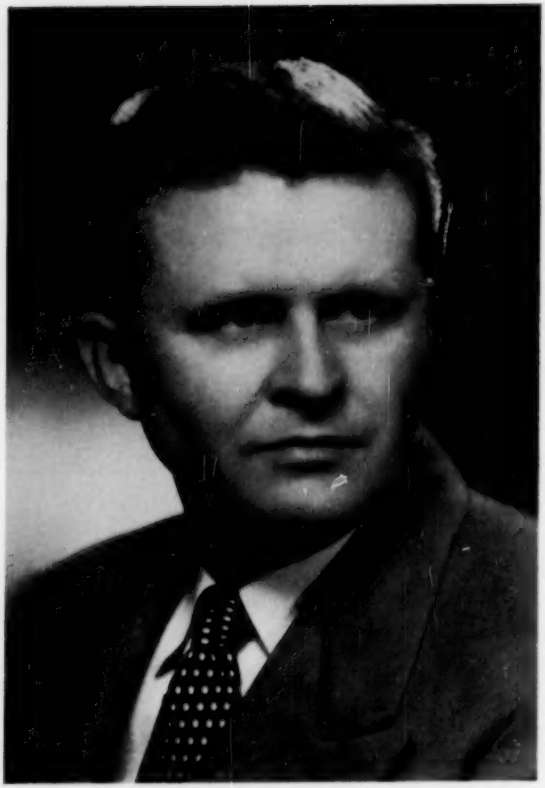

LELAND SHANOR

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SOME OBSERVATIONS AND COMMENTS ON THE LABOULBENIALES [1]

LELAND SHANOR

A little over a century has passed now since Robin and Montagne named the first species of the fungi which we classify today as belonging to the order Laboulbeniales. In 1853, in Robin's classical treatise on the natural history of vegetable parasites, these authors selected the name *Laboulbenia* for the new genus which was proposed at that time. Over four hundred additional species have been discovered since that have been assigned to it, and approximately one hundred and twenty-five other genera have been recognized also. These genera are distributed usually among three families. Only two species were described, named and figured in Robin's account. Today about 1500 species are known, making the Laboulbeniales one of the larger orders in the pyrenomycete series of the ascomycetes.

Although Robin and Montagne were the first to recognize that these minute organisms which they described belonged to the fungi, others, in the decade prior to the publication of the diagnosis and name, had seen the objects these authors reported. As might be expected, entomologists were the first to take note of these tiny bodies, but Rouget, Follin, and other early observers either looked upon them as abnormal structures belonging to the insect or regarded them as merely unidentifiable plant parasites whose relationship to known plants was not apparent. The first of the Laboulbeniales observed were detected on Coleoptera, the ground beetles, but representatives of the order now are known to infest

[1] Presidential address, Mycological Society of America. Presented September 6, 1954, at the Society meetings in Gainesville, Florida.

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representatives of not only almost all of the orders of insects but also some of the Acarina or mites.

When considering possible topics that I might discuss on this occasion, some comment upon the Laboulbeniales seemed to me to be appropriate. These are fascinating little organisms and, in spite of the reasonably large number of species that are known, I would be surprised if very many of you have seen actual specimens. Relatively few have been distributed for use as demonstration material for mycology classes, specimens are not too generally found in mycological herbaria, and relatively few mycologists seem to have had the good fortune to obtain any representatives of the Laboulbeniales from insects collected in a search for these parasites. In my own case it was not until 1943, eight years after I first became interested in the fungi, that I studied my first representative of the order. This was a species of the genus *Rickia* which I found on a museum specimen of a small insect. Five years later, in 1948, I observed my first living material, in this case a species of the genus *Laboulbenia*. In the half dozen years that have followed, I have collected and studied not only additional species that belong to these two genera but also species representing many other genera as well.

Only very brief comment on the history of the study of Laboulbeniales is necessary at this time. In the 40 years that followed the publishing of the generic name *Laboulbenia* by Robin and Montagne, several other fungi of a similar habitat and structure were discovered and named. In all, less then twenty species representing about five genera were known at the time Professor Roland Thaxter published, in 1890, his first paper concerning these superficial fungi occurring on the exoskeletons of arthropods. Included among those considered to be the major contributors over this period would be Peyritsch, Kolenati, Karsten, and Berlese. The observations of these investigators were concerned principally with morphology and taxonomy.

Inseparably associated with any consideration of the Laboulbeniales is the name of Professor Thaxter. It is seldom that the contributions of a single investigator have so completely dominated the knowledge of a particular group of fungi as does Thaxter the literature on Laboulbeniales. Thaxter devoted himself for a period of 40 years to the study of these fungi. His observations have provided the foundation for contributions by others during his life time and for those that have been made since, whether these contributions relate to taxonomy, morphology, host-parasite relationships, or some other area of investigation. During the period just mentioned, Professor Thaxter contributed numerous descriptive papers in which new species were presented, and he published

five large and magnificently illustrated monographs. These monographs are truly classics in the field of mycology. A sixth and final monograph, planned to include both a treatment of the large and diverse genus *Laboulbenia* and general biological considerations relating to members of the order, was in preparation at the time of Professor Thaxter's death in 1932. As Professor Weston has pointed out in 1933, the death of Professor Thaxter before this work was completed was indeed a tragedy for, with his passing, there went with him a store of general biological knowledge on the Laboulbeniales that he alone possessed. Probably only those who studied with Professor Thaxter, or who talked or corresponded with him on the subject, fully appreciate the magnitude of this loss. It seems quite unlikely that another student will have the opportunity to obtain the broad knowledge of the group that Professor Thaxter possessed. At the present time in this country it would seem that Dr. Richard K. Benjamin, among our younger mycologists, may be looked upon as most likely to provide eventually contributions on the general broad biological considerations now so sadly lacking for this order of fungi.

Following the publication of Thaxter's last monograph in 1931, the Laboulbeniales seem to have been generally neglected by mycologists in the United States until Benjamin and Shanor published a brief report of dioecism in *Laboulbenia formicarum* in 1950. Between 1931 and the present time several workers from other parts of the world were presenting their observations. These contributions have been almost exclusively taxonomic in nature, being concerned primarily with reporting and describing previously unknown species or noting extensions of the distribution or host range.

Before we consider the principal points that I wish to discuss, perhaps it would be well to pause briefly to consider, in a general way, the basic organization of fungi belonging to the Laboulbeniales. In all instances, the growth of a single thallus from a germinating spore does not attain the extensive mycelial development characteristic of the majority of other fungal groups. Indeed, many of the Laboulbeniales either develop on a definitely determinate pattern or their growth is very limited. For some, a mature individual will consist always of a small and definite number of cells and the formation of these cells, from the germination of the spore until growth has been completed, follows a rigidly fixed sequence. In general, a mature individual consists of: 1) a receptacle, attached at its base by what is called the foot; 2) appendages which arise from the receptacle; 3) female reproductive structures which almost invariably have their origin from a cell of the

receptacle and which eventually become the perithecia; and 4) male reproductive structures which are produced either from the receptacle or on the appendages. In spite of this relative simplicity, the remarkable variation within the order is amazing. For example, the receptacle may consist of only two cells in some genera but may be made up of a large and indefinite number of cells in others. When the receptacle is extensively developed, cell division may take place in several planes so that a massive structure is formed as is characteristic of *Zodiomyces, Euzodiomyces,* and some other genera, or cell division may take place only in the transverse plane so that the resulting receptacle is filamentous, as for example in *Filariomyces* and *Enarthromyces.* The foot is usually dark and generally appears simply to serve to attach the thallus to the integument. In some species a rhizoid may grow from the basal cell of the receptacle inward through the cuticle of the host and even into the body cavity. Species having rhizoids have been found almost exclusively on soft-bodied insects. Appendages are quite variable; in some forms they are simple, in others they are extensively branched. Some appendages bear antheridia, others remain permanently sterile. The female reproductive organ consists of a row of three cells which originate from a cell of the receptacle. Perithecial differences in the number of wall cells, the number of ascogenous cells, and other variations, are encountered among different genera in the order. Male reproductive organs are of three basic types, but all produce minute non-motile spermatia. In one type, the spermatia are formed exogenously from cells of the appendages in a manner much like typical conidial formation. In both of the two remaining types, the spermatia are formed endogenously in phialide-like antheridial cells. In the first of these, the antheridial cells are produced separately and are referred to as simple antheridia. In the other, antheridia are formed in a group and spermatia are discharged into a common cavity which opens to the exterior. These, then, are designated as compound antheridia. The nature of the structure producing the male cell is the character upon which the three families of the order generally recognized are separated.

Now let us consider some of the areas of investigation on the Laboulbeniales that should prove fruitful. Obviously time does not permit discussing all of these in great detail. Therefore, for convenience, I shall group my comments under a few general headings and call attention to some of the more recent work that has been reported.

I should like to comment first on taxonomy, morphology, and distributional studies. The largest number of papers published in recent years represents work in these areas. In 1908, Dr. Thaxter proposed

that the genera of the order Laboulbeniales be separated into three families: 1) the Laboulbeniaceae, to include genera in which spermatia are formed endogenously and the antheridia are of the simple type; 2) the Peyritschiellaceae, to include those genera in which spermatia are formed endogenously in compound antheridia; and 3) the Ceratomycetaceae, to include genera in which spermatia are formed exogenously. Under these families, grouping of genera into sub-families also was indicated. This remains the basic system of classification that is followed still by most mycologists and few exceptions have been taken to it. Colla, in 1934, proposed that four families should be recognized. She would divide the genera in Thaxter's Laboulbeniaceae into two families, using as the basis for separating them whether or not their species are homothallic or heterothallic, that is, monoecious or dioecious. The other two families as proposed by Thaxter were retained by Colla. That the distinction drawn on the basis of possessing exclusively homothallic or exclusively heterothallic thalli to characterize families is not a generally acceptable basis for classification at this level is clearly indicated. Numerous genera in other groups of fungi are known in which there are both homothallic and heterothallic species. The same might be expected in the Laboulbeniales. *Laboulbenia formicarum* serves to illustrate this point. Prior to 1950, none of the species of *Laboulbenia*, of which there are about 400, were known to be dioecious. Dioecism in *Laboulbenia formicarum* was reported by Benjamin and Shanor in 1950, 48 years after the species had been first recognized and named. Apparently Thaxter, who described it, had not detected the smaller male thalli of this species, for antheridia were not mentioned in his diagnosis and only female thalli were figured in a later monograph. Except for its dioecious character, *Laboulbenia formicarum* possesses all of the basic characteristics of other species of *Laboulbenia*. Available information and illustrations relating to *Laboulbenia hagenii* indicate that this species is probably dioecious also. Careful studies on the developmental morphology of still other species, where the facts as they relate to antheridia are obscure, may bring to light additional examples. It seems obvious that monoecism versus dioecism is not satisfactory for separating genera into families, or for separating otherwise similar species into different genera.

Professor Ernst A. Bessey, in his excellent textbook entitled the "Taxonomy and Morphology of Fungi," provides a key to the families of the order Laboulbeniales in which four are indicated. He retains the Laboulbeniaceae and Peyritschiellaceae as Thaxter proposed them but elevates the two subfamilies of Thaxter's family Ceratomycetaceae

to family rank. In the family Zodiomycetaceae, as indicated by the key characters, Bessey includes those genera having exogenously formed spermatia and massive receptacles, and in the family Ceratomycetaceae, in his more restricted sense, those genera having a similar type of spermatial formation but which lack massive receptacles. It does not seem to me, at least on the basis of our present knowledge, that the nature of the receptacle is of sufficient importance to be given such weight as would thus be accorded it. The way in which spermatia are formed appears still to be a more fundamental characteristic indicating relationship and genera possessing massive receptacles should be placed in the family with other genera having basically the same antheridial type. The case of the genus *Euzodiomyces* is one in point. This genus was diagnosed by Thaxter in 1900 and tentatively assigned to the Ceratomycetaceae because the characteristic massive receptacle in *Euzodiomyces* suggested to him a possible relationship to *Zodiomyces* and *Kainomyces*, both being genera in which the receptacle is massive. Cépède and Picard, a few years later, presented observations which seemed to support this tentative assignment which Thaxter had suggested. There the matter rested until 1951 when Benjamin and Shanor, on the basis of a more detailed study, possible because of the more abundant material in all stages of development available, were able to demonstrate the true nature of the antheridial structure in *Euzodiomyces*. It was found to be of the simple endogenous type. Consequently the transfer of *Euzodiomyces* from the Ceratomycetaceae to the Laboulbeniaceae, where it correctly belongs, was necessary. It is clear, therefore, that genera with very similar receptacles may have very different types of male reproductive structures. The nature of the antheridium would seem to remain the most significant structure on which families in the order can be separated.

The need for more morphological studies on representatives of all genera is very much needed. In spite of the fact that the order includes about 1500 species at the present time, less than a dozen of them have been critically studied in all stages of development. Lack of available material has been the limiting factor in many instances. Nevertheless, until such information is available, the relationships of many genera will remain somewhat in doubt. There are more than 40 genera that are monotypic. Many have been proposed on very limited material and, although generally adequate, sometimes not sufficient to permit relationships to be clearly indicated. Here is a fertile area for additional investigations.

For the mycologist who derives satisfaction from the discovery of

new fungi, extending this area of knowledge of Laboulbeniales offers almost unexcelled opportunity. Dr. Bessey very aptly pointed this out when he wrote concerning the order: "In view of the fact that of the 1,500,000 described species (and possibly 4,000,000 or so as yet undescribed) of insects only a few thousand have been examined for the presence of these parasites it seems reasonable to suppose that the number of species, genera and even families of this order may be greatly increased in the future." A few years ago when Dr. Benjamin conducted a study of the Laboulbeniales on staphylinid beetles in the United States, as yet unpublished, he discovered 40 species that had not been known previously. He recognized among the parasites removed from the collections of this one family of beetles seven genera that had not been diagnosed. A number of new species and several new genera have been reported since Thaxter's last work was published, especially by Middelhoek from Holland, by Lepesme and co-workers from France, by Colla from Italy, by Maire from North Africa, by Banhegyi from Hungary, and by others, but in lesser numbers, from other parts of the world. Obviously this area of research on Laboulbeniales is far from exhausted.

The cytology of the Laboulbeniales is largely unknown. Faull's studies on *Laboulbenia gyrinidarum* and *L. chaetophora*, published more than 40 years ago, remain the only reasonably complete studies that have been made. Cytological evidence demonstrating that spermatia are functional remains to be presented.

The specificity of some of the Laboulbeniales for certain hosts, and more remarkably for restricted locations on the exoskeletons, has intrigued many biologists. The reasons for such specificity remain unexplained for most species. Some of the Laboulbeniales have been found only on a single species of insect and appear to be as limited in host range as some of the fungi that parasitize seed plants and are obligate parasites of a single plant species. Other Laboulbeniales have been reported to occur on only closely related species within a genus or on species in closely related genera in a single family. A relatively small number of species have been reported from widely unrelated hosts. Many of the species of *Richia* have been obtained not only from beetles but also from the mites that normally infest these insects. One species of *Laboulbenia* has been reported to occur in Brazil on an ant of the genus *Eciton* of the order Hymenoptera. It occurs also on a hysterid beetle, of the order Coleoptera, which normally inhabits the nests of this ant, and has been reported from certain mites, which are not even insects but arachnids, that occur on the hysterid beetle. Maybe this same

fungus will be found living as a saprophyte in the nests of the ant, but under these conditions having a form of growth not suspected of belonging to a laboulbeniaceous fungus! Although this does not seem too likely, it is not completely outside the realm of possibility. Other parasitic fungi which have one pattern of growth in their parasitic phase on or in animals present quite different characteristics when growing as saprophytes. In such instances the relationship between these phases sometimes has been surprising but has been proved by carefully conducted experimental studies. As yet none of the Laboulbeniales has been artificially cultured.

Experimental studies to determine the host range of any of the species now looked upon as being very specific and limited in host range would be worthwhile contributions. Insects such as cockroaches should be ideal for such a study, for these insects can be maintained in the laboratory with ease. Suitable staphylinid or carabid beetles are not as easy to maintain, although the Laboulbeniales that might occur on some of these might be easier to study. Many years ago Peyritsch reported upon his experiments with *Stigmatomyces baeri* in which he placed newly hatched house flies with infested flies to determine the time required for mature parasites to develop on the newly hatched flies. Transfer from one host individual to others in this study was accomplished through direct contact, apparently the usual manner in which an infestation is spread. More recently two Swedish investigators, Arwidsson, in 1946, and Lindroth, in 1948, reported briefly on some experiments involving as hosts ground beetles of the genus *Harpalus*. The fungus used was a species of *Laboulbenia*, said to be close to *L. ophoni*, which was maintained for many months on *Harpalus melleti*, the host species on which it was found in great abundance in nature. This species of *Laboulbenia* was transferred from *H. melleti* to *H. punctatulus* and to *H. seladon* by using spore infested soil. Infested individuals of the original host, which were employed to provide contact inoculation, were found to be less effective than infected soil. All attempts to transfer this *Laboulbenia* to *Harpalus serripes* failed. It was concluded from this study that this species of *Laboulbenia* could be transferred to closely related species of *Harpalus* but not to all species of the genus. It was also concluded that, in this instance at least, the transfer of the fungus to non-infested hosts with soil was more effective than direct contact between infected and non-infected individuals.

The restricted position occupied on the host body by some of the Laboulbeniales is sometimes almost unbelievably rigid, but such position

specificity has been reported for a number of species. Why should one species be found on the dorsal surface of the host but not on the ventral, or on only one of the right legs and not on others or on those of the left side, or even more remarkably, constantly only on the distal end of the tarsal segment of only the right front leg? Thaxter and many others have remarked upon such specificity and have commented on why this might be so. Some have suggested that specificity of position might be related to availability of food materials. Thaxter, from his broad experience with the group, was more inclined to believe that such localization was more likely related to transfer of spores through some regularly recurring movements of the host. The mating habits of these hosts have been looked upon as one of the most likely. Until 1952 no one was aware of the fact that species of the Laboulbeniales not only could be remarkably localized on the exoskeleton but also might show some specificity for the sex of the host on which they are found. In 1952 Benjamin and Shanor published observations on six species of *Laboulbenia* from the ground beetle, *Bembidion picipes*. Often several species occurred on the same insect. One of these species was found on any part of the exoskeleton on either the dorsal or ventral surface and on both male and female insects. Another species was obtained from only the dorsal surface of the head, thorax and elytra of either males or females. A third species that was found on males and females had a much more restricted position, being found normally and almost without exception only on the humeral angle of the right elytron of females and only on the inner distal surface of the femur of the right front leg of males. The spores of this species are expelled from the perithecium only when pressure is applied. It seems to be clearly indicated for this species that the regularly recurring movement in mating behavior determines the localization to restricted areas of the exoskeleton. We have suggested that possibly other populations of this host may be found to have this species of *Laboulbenia* only on the left side and occupying corresponding positions, or that still others may be infested with it on both the right and the left. That there seem to be no physiological limitations on the position that may be occupied by this species is indicated by those rare instances in which individuals were found on parts of the body other than either the elytron of females or the femoral segment of the first right leg of males.

The three remaining species of *Laboulbenia* which were observed on *Bembidion picipes* exhibit both sex specificity and position specificity. Two of these species have been obtained only on males of this host, one occurring normally only on the mouth parts and the other only on the

tarsal segment of one or both of the front legs. The third one was obtained only from females, being found on the ventral side and then only on the posterior surface of the prosternum. What the normally recurring motions might be that insure such position specificity on only one sex of the host insect we have not the slightest indication. Unfortunately there is so little information available on the behavior of the majority of insects on which species of the Laboulbeniales occur that the student of these fungi is able to get few suggestions from the entomological literature. Therefore, if a student of the Laboulbeniales hopes to obtain answers as to why there is this restricted specificity of position on the host insect, he must become a keen student of insect behavior as well.

Before leaving the matter of specificity of position which some of the Laboulbeniales exhibit, I cannot very well pass over commenting briefly on that demonstrated by species of *Chitonomyces* found on the aquatic gyrinid beetle, *Orectogyrus specularis*. Here we have an example of specificity that would seem unbelievable were it not supported by careful observations which have been confirmed recently. No less than 16 distinct species of *Chitonomyces* were reported by Thaxter from this one host, and all are to be found on very definite and restricted areas on either the terminal abdominal segment or the neighboring genital lobes. So specific are these species of *Chitonomyces* for their position on this host that the identity of the species may be predicted accurately if only the place of collection and position on the host is known. When Dr. Benjamin had an opportunity in 1951 and 1952 to study the Thaxter collections at Harvard he examined a series of specimens of *Orectogyrus specularis* on which these 16 species of *Chitonomyces* had been reported. He made an additional discovery of a situation of which even Professor Thaxter apparently was not aware. Not only do these species exhibit remarkable position specificity but all but one of them seems to be specific for the sex of the host on which it is found. Dr. Benjamin summarized his findings in a personal communication to me and generously gave me permission to present his summary at this time. He reported: "Of the 16 species of *Chitonomyces* occurring on this host only one is found on both male and female insects. Six are limited to males and 9 to females. It is interesting to note that 4 of the species found on males occur on the genitalia, while none of those found on females are in any way related to the genitalia." Dr. Benjamin has surveyed several other families of aquatic beetles and has made comparable observations on this host-parasite relationship but, lacking information on the behavior habits of the hosts that might explain such specificity, has delayed publication of

his observations. When more information becomes available, he plans to report on this study in detail.

The last area offering an excellent opportunity for investigation of Laboulbeniales that I want to touch upon briefly is nutrition. Experimental studies that provide information on how these fungi obtain the substances they need for growth and development are at present lacking. The few species that are known to produce a rhizoidal structure from the basal cell of the receptacle appear definitely to absorb their nourishment from the living part of the insect within the exoskeleton to which they are attached. In certain others that have been critically examined, for example species of *Herpomyces* by Thaxter prior to 1932 and by Richards and Smith, reported at a recent meeting of the Entomological Society of America in San Francisco, very delicate rhizoids have been demonstrated which penetrate the cuticle at least to the hypoderm. Very similar structures have been seen in *Laboulbenia fasciculata* by Poisson and in young individuals of *Laboulbenia blanchardi* by Cépède. For those Laboulbeniales in which no such structures have been detected, and for which the foot appears to serve principally to anchor the individual to the cuticle, Thaxter believed that the absorption of nutritional substances through the foot membrane was likely. Cavara advanced the opinion that, for most species of the order, the foot serves merely for attachment and that absorption of nutritional materials must occur through the walls of the appendages rather than through the foot. Thaxter took vigorous exception to this suggestion, pointing out that most species do not come in contact with water except for the small amount that might condense on the surface of the host. He could not imagine how these fungi could obtain a sufficient food supply, under these circumstances, from their environment. Thaxter pointed out also that the entire individual is covered by an almost impermeable membrane which would make absorption over the surface almost impossible. This covering is even highly impervious to the usually rapidly penetrating substances such as are used generally for the killing and fixing of material for cytological studies. Thaxter regarded as highly significant, also, the remarkable specificity of these fungi, in most cases, for only one or only a few host species. This, he argued, would indicate a parasitic rather than a saprophytic type of life.

Other suggestions attempting to explain the possible nutrition of members of the order have been proposed also. Picard, for example, expressed the opinion that possibly the nutrition of the Laboulbeniales is derived from the waxy material which covers the body of all insects

and which is secreted in a liquid state. The presence of tegumentary glucose has been demonstrated among arthropods generally.

None of the Laboulbeniales have been grown in artificial culture. That the spores of at least some species will germinate without contact with a host is clear. In several instances the development of the spore, at least through the formation of the indurate foot, has been observed within the perithecium when spores have been retained within it abnormally. Also, we have observed on several occasions the germination of spores that have become attached to the perithecium from which they have been discharged but growth beyond the four-celled stage has not been seen. Perhaps, unless a contact with living cells of the host is made by the time this stage is reached, the individual can develop no further. The successful cultivation of any of the Laboulbeniales on artificial media would open the way for a wide variety of experimental studies. Then it would be possible to carry out with greater ease cross inoculation studies involving the morphologically similar species that occur on different hosts and which are now separated largely on that basis. Then it will be possible to clarify some of the taxonomic problems that now involve certain species complexes in the order. Also it would be more feasible to study more effectively phenomena such as position specificity and sex specificity than is possible at the present time. It would seem, therefore, that the key to obtaining a substantially broader understanding of the Laboulbeniales than we now have will depend largely upon solving the problem of how to grow at least some members of the order apart from their insect hosts. If all of the Laboulbeniales are obligate parasites, this may not be accomplished. However, if some of them derive their nutrition only from the cuticular substances or secretions, then laboratory cultivation on artificial media may be possible.

The Laboulbeniales offer an unusual challenge!

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THE NUTRITION OF SCHIZOSACCHAROMYCES POMBE

ILDA MCVEIGH AND EVERETT BRACKEN

(WITH 4 FIGURES)

Yeasts have been, for almost a century, the bases of investigations leading to fundamental discoveries which have become the foundation upon which much of modern biological science has been built. According to R. J. Williams (**1941**), "Some of the most far-reaching discoveries in the whole field of biology have been made in connection with the study of yeasts." The object of this investigation was to devise a complete synthetic medium, consisting of known quantities of chemically defined substances, in which the yeast *Schizosaccharomyces pombe* Y-658 grows optimally. It is hoped that this contribution may aid in further metabolic studies.

MATERIALS AND METHODS

Grateful acknowledgment is made to Dr. L. J. Wickerham of the Northern Regional Research Laboratory, Peoria, Illinois, for the culture of *Schizosaccharomyces pombe* Y-658 which was used throughout this investigation. Stock cultures of the organism were carried on agar slants composed of the complete medium plus 1.5% agar and 0.3% yeast extract.

The minimal medium contained the following compounds per liter of solution: dextrose, 50.0 g; KH_2PO_4, 1.5 g; $MgSO_4.7H_2O$, 0.5 g; asparagine, 2.0 g. To this solution the minor mineral elements were added in parts per million as follows: B, 0.01; Cu, 0.04; Fe, 0.2; Mn, 0.02; Mo, 0.02; and Zn, 0.18. The pH of the medium was 5.5–6.0.

The complete medium consisted of the minimal medium, the vitamin, the purine and pyrimidine, and the amino acid supplements. The supplement of B vitamins contained sufficient amounts to give the following concentrations of each per liter when 10.0 ml were used per liter of medium: thiamine HCl, 0.6 mg; biotin, 15.0 μg; pteroylglutamic acid (PGA), 1.5 mg; riboflavin, 0.6 mg; pyridoxine HCl, 0.6 mg; niacin, 0.6 mg; p-aminobenzoic acid (PABA), 0.6 mg; Ca pantothenate, 0.6 mg; and inositol, 100.0 mg. The purine-pyrimidine supplement, which contained adenine, guanine, xanthine, and uracil, was added to the basal

medium in sufficient amounts to supply 10.0 mg of each base per liter. The bases were dissolved by prolonged heating in distilled water made alkaline by the addition of NaOH. The pH was then adjusted to approximately 7.0, and the solution was made to volume. The amino acids were supplied in the form of a supplement which contained 50.0 mg of acid-hydrolyzed "vitamin-free" casein and 250.0 μg each of cystine and tryptophane per ml. Twenty ml of this supplement were used per liter of medium. The casein hydrolyzate was a product of the Nutritional Biochemicals Corporation.

The inoculum was prepared by transferring a small quantity of cells from a 24-hour stock culture to a liquid medium from which the particular substance or substances concerned in the experiment had been omitted. This was incubated for 12 hours at 30° C, centrifuged and the supernatant decanted; the cells were then washed three times in physiological saline solution and finally resuspended in the latter. One drop of this suspension was used per tube of medium. All cultures were incubated at 30° C.

Growth was measured turbidimetrically by use of a Klett-Summerson colorimeter (No. 42 filter). To each tube, containing 9.0 ml of culture solution, 4.5 ml of 95 percent ethanol were added, and the tube was shaken vigorously just prior to measuring the turbidity. The alcohol decreased the foaming and flocculation of the yeast. The turbidity values shown in the tables and graphs are the averages of four or more determinations from which the reading of the uninoculated control medium had been subtracted.

RESULTS

Nature of the Growth Factor Requirements

The initial phase of the experimental work was designed to determine whether or not *Schizosaccharomyces pombe* Y-658 requires factors other than those contained in the minimal medium and, if so, the nature of

TABLE I

EFFECTS ON THE GROWTH OF S. POMBE Y-658 CAUSED BY THE ADDITION OF VARIOUS SUPPLEMENTS TO THE MINIMAL MEDIUM

Medium	Turbidity
Minimal medium	0
Minimal medium + vitamin supplement	127
Minimal medium + vitamin supplement + amino acid supplement	182
Minimal medium + amino acid supplement	0
Minimal medium + amino acid supplement + vitamin supplement + yeast extract	303

these requirements. The media used and the turbidity produced in each during 48 hours of incubation are indicated in Table I. The yeast failed to grow in those media which lacked the vitamin supplement. When the only addition to the minimal medium was the solution of vitamins, the turbidity produced during the period of growth was 40% of the maximum which occurred in the medium that contained not only the vitamins but also amino acid supplement and yeast extract. With the amino acids in addition to the vitamins, growth was about 60% of the maximum.

Table II

The effects of yeast extract and of a combination of purine and pyrimidine bases on the growth of S. pombe Y-658. The purine-pyrimidine supplement included adenine, guanine, xanthine, and uracil

Medium	Turbidity
Minimal medium	0
Minimal medium + vitamin supplement + amino acid supplement + adenine, guanine, xanthine, and uracil	295
Minimal medium + vitamin supplement + amino acid supplement + yeast extract + adenine, guanine, xanthine, and uracil	298

In a supplemental experiment (Table II) performed under similar conditions, it was found that the inclusion of yeast extract in the minimal medium which had been supplemented with the vitamins, amino acids, and the four bases—adenine, guanine, xanthine, and uracil—resulted in no better growth than occurred in the medium without it.

Vitamin Requirements

Measurements were made of the turbidities produced after 48 hours of incubation in a series of media complete except for the omission of one of the vitamins, a different one from each medium. At the same time, the yeast was inoculated into the minimal medium; the complete medium; a medium complete except for the omission of both PGA and PABA; and one which differed from the complete by having 30.0 μg/ml of β-alanine substituted for the pantothenic acid. Growth in the absence of riboflavin, niacin, PGA, or PABA, and in the absence of both PGA and PABA did not differ significantly from that in the complete medium (Table III). Without thiamine, growth was reduced to about 50% of the maximum, and without pyridoxine it was approximately 35%. Little, if any, growth occurred in the media lacking biotin, pantothenic acid, or inositol. The substitution of β-alanine for pantothenic acid in the complete medium resulted in no significant change in the amount of growth of the yeast.

TABLE III

GROWTH OF S. POMBE Y-658, AFTER 48 HOURS, IN THE ABSENCE OF VARIOUS VITAMINS

Medium	Turbidity
Minimal medium (Control No. 1)	0
Complete medium (Control No. 2)	324
Complete medium—PGA and PABA	322
Complete medium—PABA	319
Complete medium—PGA	310
Complete medium—riboflavin	325
Complete medium—thiamin	161
Complete medium—niacin	304
Complete medium—pyridoxine	112
Complete medium—biotin	5
Complete medium—inositol	3
Complete medium—pantothenic acid	2
Complete medium—pantothenic acid + 30 μg./ml. β-alanine	314

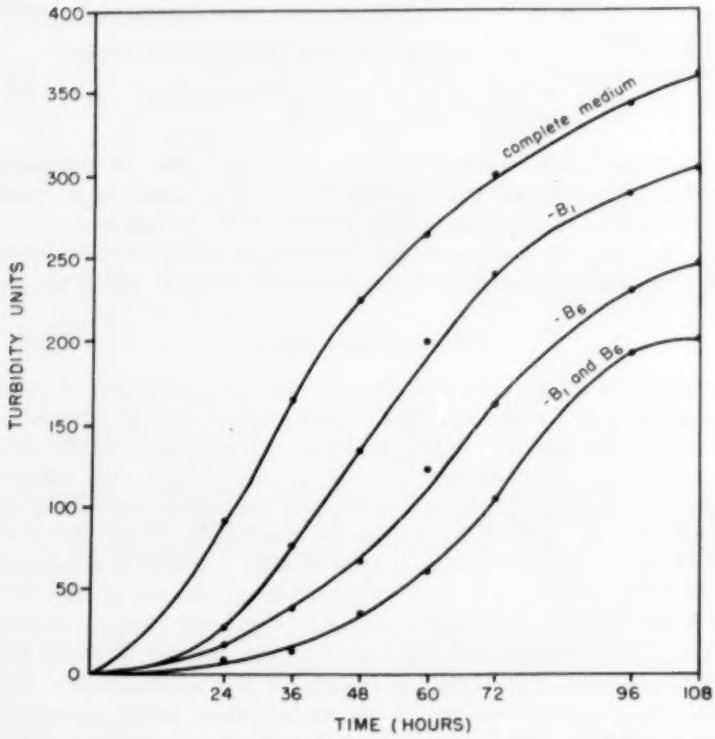

FIG. 1. Growth curves of *S. pombe* Y-658 in the complete medium, thiamine-deficient medium, pyridoxine-deficient medium, and in the medium lacking both thiamine and pyridoxine.

To determine whether thiamine in the absence of pyridoxine has an inhibitory effect on the growth of *S. pombe* Y-658, and, if not, whether this yeast is able to overcome the partial deficiencies for these two vita-

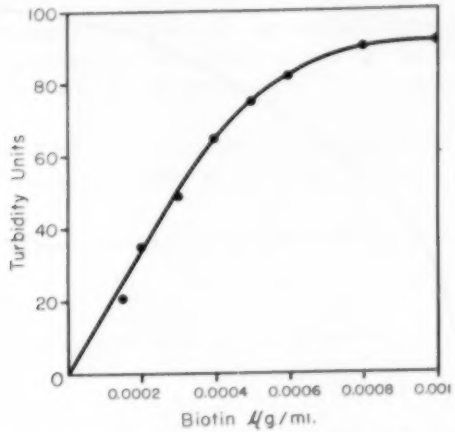

Fig. 2. Graph showing the effect of increasing amounts of biotin on the growth of *S. pombe* Y-658 in minimal medium plus the other necessary vitamins.

mins, observations were made of its growth in the complete medium, this medium lacking both vitamins, and in the same medium from which thiamine and pyridoxine, respectively, had been omitted (Fig. 1). Best growth occurred when both vitamins were available and least when

Table IV

Effect of increasing amounts of pantothenic acid on growth of S. pombe Y-658 in the minimal medium supplemented with amino acids, purine and pyrimidine bases, biotin, thiamine, pyridoxine, and inositol

Pantothenic acid, µg/ml	Turbidity
0.0	0
0.001	8
0.01	22
0.05	281
0.1	301
1.2	302

both were lacking. The omission of pyridoxine from the otherwise complete medium resulted in less growth than occurred when only thiamine was omitted.

In a preliminary experiment, the amount of biotin required for maximal growth of *S. pombe* Y-658 was found to be not more than

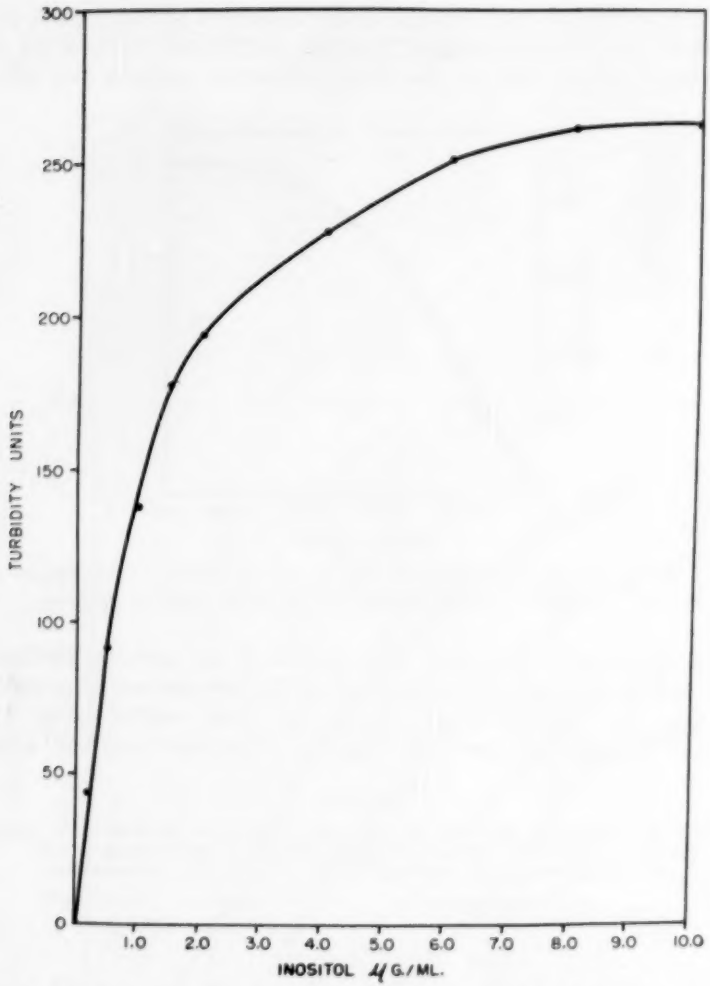

FIG. 3. Graph showing the effect of increasing amounts of inositol on the growth of *S. pombe* Y-658 in an otherwise complete medium.

0.001 μg/ml. In a subsequent experiment (FIG. 2) measurements were made of the response of the yeast to increments of biotin after 48 hours of incubation in the minimal medium supplemented with thiamine, pyridoxine, inositol, and pantothenic acid. An increase in growth from none, in the absence of biotin, to a maximum at a concentration of

0.001 μg/ml occurred. The amino acid supplement and the purine and pyrimidine bases were omitted from the basal medium used in this experiment in order to avoid the possibility of introducing contaminating traces of biotin; these omissions account for the low maximum growth which was obtained. However, the amount of biotin found optimum for growth in this medium was sufficient also for the higher maximum growth which occurred in the presence of these supplements (Tables V, VI).

By a similar procedure, the optimal concentration of inositol for the growth of the yeast was found to be 8.0 μg/ml (Fig. 3), and that of

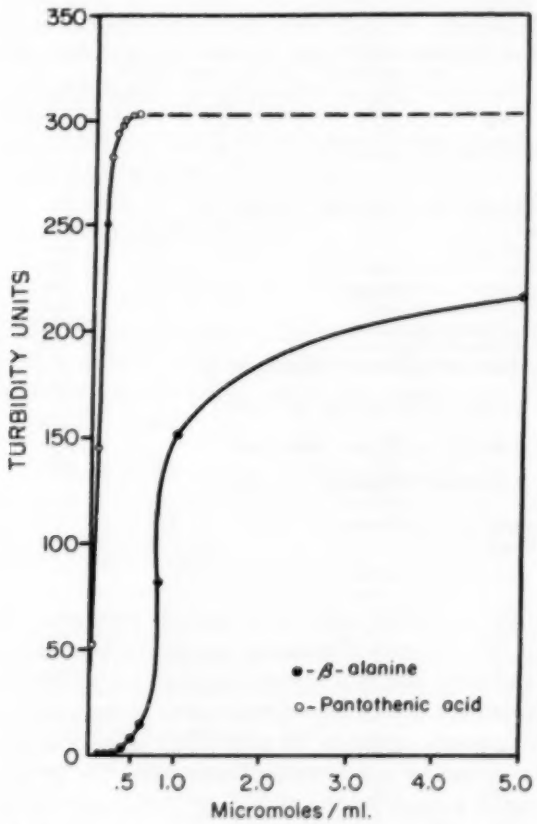

Fig. 4. Graph showing effects of equimolecular concentrations of pantothenic acid and of β-alanine on the growth of *S. pombe* Y-658 in otherwise complete media.

pantothenic acid 0.1 μg/ml (TABLE IV). The growth was almost linear with increments of pantothenic acid between 0.0 and 0.05 μg/ml; with higher concentrations, there was little increase in growth.

Next, a comparison was made of the response of *S. pombe* Y-658 to equimolecular concentrations of pantothenic acid and β-alanine, a precursor of the former. The turbidities were measured after 48 hours of incubation (FIG. 4). Growth increased sharply with the increase of pantothenic acid to 0.25 micromoles (0.0645 μg)/ml and reached a maximum at approximately 0.3 micromoles/ml. At these concentrations, β-alanine was ineffective, but, with increments between 0.6 and 1.0

TABLE V

EFFECT OF THE ADDITION OF VARIOUS COMBINATIONS OF SUPPLEMENTS ON THE GROWTH OF S. POMBE Y-658

Supplements added to minimal medium +required vitamins	Turbidity		
	36 hours	48 hours	96 hours
Amino acid supplement + adenine, guanine, xanthine, and uracil	122	289	329
Amino acid supplement	72	163	293
Adenine, guanine, xanthine, and uracil	53	154	251
Amino acid supplement + adenine	92	204	310
Amino acid supplement + uracil	90	197	317
Amino acid supplement + xanthine	75	161	308
Amino acid supplement + guanine	76	165	304
Amino acid supplement + guanine, uracil, and xanthine	95	173	315
Amino acid supplement + adenine, guanine, and uracil	106	278	329
Amino acid supplement + adenine, uracil, and xanthine	101	270	323
Amino acid supplement + adenine, guanine, and xanthine	94	179	321
Amino acid supplement + adenine and uracil	104	279	328
Adenine and uracil	70	151	255

micromoles/ml, a marked increase in growth occurred. Further increases in the concentration of β-alanine resulted in a much more gradual increase in turbidity, and at a concentration of 5.0 micromoles/ml—the highest amount used in this experiment—growth equivalent to that obtained with optimum amounts of pantothenic acid was not attained. However, in an earlier experiment (TABLE III), the growth obtained with β-alanine at a concentration of 30.0 μg (116.2 micromoles)/ml was not significantly different from that which occurred in the complete medium with pantothenic acid supplied in optimum amounts.

Purine and Pyrimidine Bases

It was found in the early experiments (Tables I, II) that the purine and pyrimidine bases—although not essential for the growth of the yeast—did exert a stimulatory effect. To determine which of the four bases, used earlier, caused the stimulation, the yeast was grown in the minimal medium containing optimal amounts of the required vitamins and the various combinations of supplements listed in Table V. Without the bases or with either xanthine or guanine included in the medium supplemented with the required vitamins and the amino acids, growth after 36 hours was approximately 60% of that in the medium to which the four bases had been added; with the addition of either adenine or uracil, growth was approximately 75% of that in the complete medium. A still greater stimulation occurred when both adenine and uracil were supplied. However, after 36 hours, the best growth was in the complete medium which contained the four bases. After a longer period of incubation (96 hours), growth in the absence of the bases approached that in their presence, while that in the medium which contained the essential vitamins, the amino acid supplement, and both adenine and uracil was equal to that in the complete medium.

Sources of Nitrogen

The preliminary experiment (Table I) showed that, although *S. pombe* Y-658 is capable of growing with asparagine as its sole source of nitrogen, better growth occurs when the amino acid supplement is

Table VI

Effect of various sources of nitrogen on the growth of S. pombe Y-658

Supplements added to a basal medium* + required vitamins	Turbidity		
	48 hours	72 hours	120 hours
No supplement	132	160	160
$(NH_4)_2SO_4$ + asparagine	153	162	164
$(NH_4)_2SO_4$ + asparagine + adenine, guanine, xanthine, and uracil	163	171	183
$(NH_4)_2SO_4$ + asparagine + amino acid supplement + adenine, guanine, xanthine, and uracil	233	241	241
$(NH_4)_2SO_4$ + glutamic acid + adenine, guanine, xanthine, and uracil	203	203	213
$(NH_4)_2SO_4$ + arginine + adenine, guanine, xanthine, and uracil	184	195	204

* The basal medium used for this experiment differed from that used in the previous experiments by having $(NH_4)_2SO_4$ (2 gm/l) substituted for asparagine and 20.0 gm of dextrose per liter instead of 50.0 gm.

added to the asparagine medium. After 36 hours of incubation in the minimal medium into which the optimum amounts of the required vitamins and the purine-pyrimidine supplement had been incorporated, the turbidity was approximately 43% of that in the medium which contained also the amino acid supplement (TABLE V). After 96 hours, the difference was not so great; the growth in the former medium was 76% of that in the latter.

A further experiment (TABLE VI) indicated that *S. pombe* Y-658 can utilize $(NH_4)_2SO_4$ as its sole source of nitrogen. After 48 hours of incubation in a medium with NH^-_4 as the nitrogen source, the turbidity was 132 as compared to 153 in a similar medium which contained asparagine in addition to the ammonium compound; there was no significant difference in the turbidity produced in these two media in 72 hours. Growth was improved slightly by the inclusion of the purine-pyrimidine supplement in the medium containing both $(NH_4)_2SO_4$ and asparagine, and it was improved even more by the further inclusion of the amino acid supplement. The substitution of arginine for asparagine in the medium containing $(NH_4)_2SO_4$ and the purine-pyrimidine supplement resulted in an increase in the growth of the yeast; a greater increase occurred when glutamic acid was used as the substitute.

DISCUSSION

It is interesting to note that *Schizosaccharomyces pombe* Y-658, unlike the strain (or strains?) investigated by Burkholder *et al* (**1944**) and by Schultz and Atkin (**1947**), is not deficient for niacin. However, these strains were alike in requiring biotin, inositol and pantothenic acid. Another strain (Y-164) with which some preliminary comparative studies were made during the course of this investigation also is deficient for niacin as well as for the three vitamins just mentioned. The strain used by Emery *et al* (**1946**) was similar to, if not the same as, Y-658 since it, also, required biotin, inositol and pantothenic acid but did not require niacin.

Evidently *S. pombe* Y-658 has a partial deficiency for both thiamine and pyridoxine since each must be available in order to obtain a maximal growth rate. It should be noted, however, that the inclusion of either of the vitamins in the medium resulted in a significant increase in growth over that occurring when both vitamins were lacking. There was no evidence of inhibition by thiamine in the absence of pyridoxine as has been reported by Schultz *et al* (**1940**), Schultz and Atkin (**1947**) and Rabinowitz and Snell (**1951**) for some members of the genus *Saccharo-*

myces. The relationship between the two vitamins in the metabolism of *S. pombe* probably is somewhat similar to that described by Moses and Joselyn (1953). They reported that the two vitamins "served as functional substituents for each other" in the nutrition of *Saccharomyces cerevisiae* 6044. They also noted that both vitamins were indispensable for a maximal rate of growth.

Emery *et al* (**1946**) suggested the use of *S. pombe* for the assay of inositol but did not indicate the strain. Strain Y-658, which was used in this investigation, is very sensitive to inositol and shows a very good response over a range of concentrations from 0.0 to 8.0 μg/ml, inclusive. However, because of flocculation, it is difficult to obtain accurate turbidimetric readings, and, for this reason, it is not recommended for assay purposes.

Wickerham (**1946**) reported that many yeasts are capable of assimilating $(NH_4)_2SO_4$, urea or asparagine when supplied adequate amounts of vitamins. *S. pombe* Y-658 grows better in the presence of vitamins when supplied with nitrogen in the form of arginine, glutamic acid, or a mixture of the amino acids than with either $(NH_4)_2SO_4$ or asparagine (Tables I, VI). However, fair growth does occur with either of the latter two as nitrogen sources; therefore, it has no requirement for a specific amino acid.

The phenomenon of flocculation of *S. pombe* Y-658, which was of particular significance in this investigation because it seriously hampered preparation of uniform inocula and interfered with accurate measurements of turbidity, is of sufficient importance to warrant discussion. This yeast characteristically grows in hard, flaky clumps, which are difficult to disperse into a homogeneous suspension of individual cells by ordinary methods. Conversely, a closely related strain *S. pombe* Y-164 grows in soft, creamy colonies which readily go into uniform and rather stable suspensions.

Flocculence, at least in certain yeasts, has been found to be a genetic characteristic (Pomper and Burkholder, **1949**; Thorne, **1952**), and the presence of bivalent or polyvalent ions has been shown (Jansen and Mendlik, **1951**) to increase the tendency to flocculate. The latter fact suggests that flocculation is associated with the electrokinetic properties of the cell wall. $MgSO_4$, which was incorporated in the basal medium, undoubtedly increased the tendency of the yeast to flocculate. This was borne out by the fact that flocculation decreased noticeably when the cells were washed and suspended in the presence of Na^+ ions contained in the physiological saline solution. In the course of trying various substances as anti-foaming and anti-flocculating agents, the use of 95% ethanol for

this purpose was more or less accidentally discovered. A concentration of one part of alcohol to two parts of culture solution appeared to be rather critical; flocculation was not decreased by either greater or lesser amounts of alcohol. This suggests the possibility that the cells have a gelatinous or mucoid coating which ordinarily binds them firmly together and that the proper concentration of alcohol tends to dissolve this coating. A low concentration fails to accomplish this dissolution, whereas too high a concentration congeals or coagulates the material.

SUMMARY

Schizosaccharomyces pombe Y-658 is deficient for biotin, pantothenic acid, and inositol. The following amounts per ml are sufficient for maximal growth: biotin, 0.001 μg; pantothenic acid, 0.1 μg; inositol, 8.0 μg. The inclusion of either thiamine or pyridoxine in the medium resulted in a significant increase in growth over that which occurred in the absence of both. For a maximal growth rate, both were essential.

Adenine and uracil had a stimulatory effect on the early growth of the yeast.

Although *S. pombe* Y-658 utilized either $(NH_4)_2SO_4$ or asparagine as its sole source of nitrogen, its growth was stimulated by glutamic acid, by aspartic acid, and even more, by vitamin-free casein hydrolyzate.

A method of overcoming flocculence is described.

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THE EFFECTS OF HUMIDITY, TEMPERATURE AND CARBON DIOXIDE ON SPORULATION OF CHOANEPHORA CUCURBITARUM [1]

H. L. Barnett and Virgil Greene Lilly

Under the usual laboratory conditions in test tubes, flasks or tightly closed Petri dishes, the high humidity and poor ventilation create abnormal conditions which may permit growth of the mycelium, but often greatly reduce sporulation of many fungi. This may be due to unfavorable humidity, insufficient oxygen or to an accumulation of carbon dioxide or other gases resulting from the metabolism of the fungus.

Comparatively few studies of the effects of the conditions of the atmosphere in the culture vessels have been made, but it is known that these factors are important for some fungi. Henry and Andersen (**1948**) have shown that an accumulation of ammonia reduces sporulation of *Piricularia oryzae,* and Bright *et al* (**1949**) reported that an increase in carbon dioxide reduced sporulation of *Saccharomyces cerevisiae.*

Ternetz (**1900**) found that high relative humidity was necessary for the formation of apothecia of *Ascophanus carneus.* Klebs (**1900**) showed that the optimum relative humidity for zygospore formation of *Spordinia grandis* was higher than the optimum for the production of sporangia. Only zygospores were formed near the saturation point of the atmosphere. Relative humidity had some effect on the production of perithecia and conidia of two species of *Magnusia* (Sweet, **1942**), but the two species varied in their response. One species produced perithecia at a relative humidity too low for the formation of conidia. On the other hand, Goldring (**1936**) found that relative humidity had little or no effect on the relative numbers of sporangia and sporangiola produced by two strains of *Blakeslea trispora.*

No reference to work on the effect of humidity on *Choanephora cucurbitarum* was found, although a study of light, temperature and nutritional factors that influence sporulation was reported by Barnett and Lilly (**1950**). This paper reports the results of continued studies of environmental conditions affecting sporulation.

[1] Published with the approval of the Director of the West Virginia Agricultural Experiment Station as Scientific Paper No. 488.

MATERIALS AND METHODS

The isolate used in this study was obtained from a diseased squash flower at Morgantown, West Virginia in 1947. The medium was the same used in the previous study (Barnett and Lilly, **1950**), except that Difco Casamino Acids, 2 gm per liter, replaced asparagine as the nitrogen source, and the amount of glucose was increased from 2 to 3 gm per liter.

Cultures were grown in Petri dishes containing approximately 25 ml of agar medium for two days, under continuous fluorescent light, in order to permit growth of mycelium under uniform conditions, but prevent sporulation. After this time, the cultures were placed in desiccators and the lids of the Petri dishes were removed to permit free circulation of gases around the fungus. The desiccators and cultures were then placed in total darkness at the desired temperature for 12–16 hours, during which time sporulation occurred. This procedure made certain that the variable conditions were effective only during the sporulation process.

The accumulation of carbon dioxide in the desiccators was prevented when desired by placing an open dish of 5% KOH solution in the bottom. In some experiments, carbon dioxide was added to the desiccators after partial evacuation. A saturated atmosphere was maintained in the desiccators by wet paper towels. Approximately 50% relative humidity was maintained by a saturated solution of ammonium nitrate. Uncovered Petri dish cultures placed beside the desiccators served as controls. Uniform temperatures were maintained by the use of a constant temperature room at 25° C and water-jacketed incubators for 28° and 30° C.

The numbers of conidial heads and sporangia in a culture were estimated by counting each in a given fraction of the area of the culture and calculating the total. Since variation in numbers occurred in different trials, the ranges of the totals are used in the tables. All experiments were repeated several times.

EXPERIMENTAL RESULTS

Preliminary experiments showed that culturing the fungus in tightly closed Petri dishes or desiccators reduced the numbers of both sporangia and conidial heads. On the assumption that this might be due to the accumulation of carbon dioxide, experiments were carried out to test this hypothesis. Briefly, the results of several repetitions of these experiments were as follows: (1) open Petri dishes in closed desiccators,

carbon dioxide allowed to accumulate, 0 to 25 conidial heads and 0 to 10 sporangia per culture; (2) open Petri dishes in closed desiccators, carbon dioxide removed by a 5% solution of KOH, 100 to 500 conidial heads and 1000 to 2000 sporangia; (3) carbon dioxide added to closed desiccators, no sporulation.

All subsequent experiments on the effects of humidity and temperature were carried out in desiccators containing dishes of a 5% solution of KOH to remove the carbon dioxide. The results, summarized in TABLE I, show the range in numbers of conidial heads and sporangia in several repetitions.

TABLE I

EFFECTS OF TEMPERATURE AND HUMIDITY ON THE NUMBER OF CONIDIAL HEADS AND SPORANGIA PRODUCED BY *Choanephora cucurbitarum*

Temperature	Relative humidity	No. conidial heads	No. sporangia
25° C	Control	1500–3000	Less than 50
25° C	50%	1000–1500	1200–1500
25° C	100%	Less than 200	2000–4000
28° C	Control	800–1200	Less than 200
28° C	50%	Less than 300	500–1000
28° C	100%	Less than 100	1000–4000
30° C	Control	500–1000	Less than 20
30° C	50%	None	1000–2000
30° C	100%	None	1000–1500

DISCUSSION AND SUMMARY

The principal conclusions to be drawn from these experiments are as follows: (1) An accumulation of carbon dioxide in closed culture vessels reduces or prevents sporulation; (2) Both temperature and relative humidity are active in determining the numbers of conidial heads and sporangia formed; (3) The predominance of conidial heads or sporangia is governed largely by the relative humidity and its effect is magnified at temperatures above 25° C. A relative humidity approaching 100% favors the production of sporangia, while low humidity favors the formation of conidial heads.

The asexual sporulation of *Choanephora cucurbitarum* has been shown in this and in previous studies to be greatly influenced by temperature, relative humidity and the amount of carbon dioxide in the atmosphere, as well as exposure to both light and darkness, and by nutritional factors.

Few, if any, of the fungi studied in culture have been so sensitive to differences in the environment. This would indicate a high degree of

adaptation of the fungus to its particular mode of life. In nature, *C. cucurbitarum* is a weak parasite, attacking and rotting succulent flowers and young fruits of cucurbits and other plants. The formation of conidia, which is the common reproductive stage found in nature, occurs during the second or third night after infection. It seems probable that the greatest effect of humidity is during the first few hours of darkness, early in the formation of the spore-bearing structures. If this assumption is correct, in order to find sporangia in nature, one should look early in the morning following a night which is hot and humid during the early hours.

These results suggest that other species of Mucorales having two forms of sporulation should be investigated to determine if they are affected by humidity. The fact that *Blakeslea trispora* (**3**) showed little response to differences in relative humidity indicates that closely related species may differ widely in this respect. The response to changes in the environment is more likely due to adaptation to a specific habitat rather than to a phylogenic relationship.

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BIOLOGICAL ACTIVITY OF *p*-METHOXY-TETRACHLOROPHENOL [1]

MARJORIE ANCHEL, ANNETTE HERVEY AND WILLIAM J. ROBBINS

Drosophilin A, an antibiotic compound from *Drosophila subatrata* (**5**), was identified as *p*-methoxy-tetrachlorophenol (**1**).[2] Since chlorophenols (e.g. pentachlorophenol) and chloroquinones (e.g. tetrachloroquinone, active ingredient of "spergon" and 2,3-dichloro-1:4-naphthaquinone, active ingredient of "phygon") are used as antifungal agents (**3**), it was considered of interest to compare the antibiotic activity of *p*-methoxy-tetrachlorophenol with a number of related phenols and quinones.[3]

The results of tests with bacteria and fungi are presented in TABLE I. All test samples were in 20 percent ethanol. Assays were made by serial dilution in liquid media using complete inhibition of growth, as macroscopically observed, for the end point. *Mycobacterium smegma* was grown in modified Kirchner's medium; all other bacteria in a beef extract broth (**4**). The pH of these media was 6.6 to 6.8 after sterilization. For the fungi a medium of mineral salts, dextrose, and peptone at pH 6.0 was employed (**2**). Tests on the fungi and on *M. smegma* were made in 1 ml quantities of liquid, for the other bacteria 0.5 ml volumes were used. *Trichophyton mentagrophytes, Bacillus subtilis*

[1] This investigation was supported in part by a research grant (E-226) from the National Microbiological Institute of the National Institutes of Health, Public Health Service.

[2] The statement made in this paper that "Drosophilin A is believed to be the first antibiotic compound isolated which contains a halogenated benzene ring," was due to an oversight. Several other antibiotic compounds with a chlorinated benzene ring were isolated earlier, e.g. geodin, erdin, griseofulvin, diploicin, and more recently, aureomycin.

[3] Source of compounds tested:

Compounds Nos. 2, 5, 6, 7, 8, 13 and 14 were obtained from Eastman Kodak Co., compound No. 1 from the Amend Drug and Chemical Co., and compound No. 15 from the Naugatuck Chemical Co.

Compounds No. 3 and No. 10 were synthetic samples, prepared according to Thiele and Winter (**8**). The melting points were for No. 3, 145° and for No. 10, 130–3°.

Compound No. 4 was isolated from culture liquids of *Lentinus degener* (**2**).

Compounds No. 9 and No. 11 were prepared by reduction of the corresponding quinones No. 2 and No. 4 respectively, with SO_2. The melting points (124° and 126°) agreed with those reported in the literature (**7**), (**6**).

Compound No. 12 was isolated from culture liquids of *Drosophila subatrata* (**5**, **1**).

TABLE I

MINIMUM INHIBITORY CONCENTRATION OF PHENOL AND QUINONE DERIVATIVES IN MICROGRAMS PER ML
(The letter "p" signifies partial inhibition.)

	1 benzoquinone	2 toluquinone	3 5-hydroxy -toluquinone	4 5-methoxy -toluquinone	5 tetrachloroquinone (spergon)	6 hydroquinone	7 tetrachloro -hydroquinone	8 p-methoxyphenol	9 tolahydroquinone	10 5-hydroxy -tolahydroquinone	11 5-methoxy -tolahydroquinone	12 p-methoxy -tetrachlorophenol	13 pentachlorophenol	14 pentabromophenol	15 2-3 dichloro 1-4 naphthaquinone (phygon)
BACTERIA															
Bacillus subtilis	4	8	125	8	31	125	16	250	16	63	8	31			
Bacillus mycoides	4	4	125	8	31	125	16, 8p	500	31	63	16	63			
Escherichia coli	31	31	125	63	63	125	125	250	125	125	31	125			
Klebsiella pneumoniae	31	31	250	31	125	250	125	250	125	125	31	250			
Mycobacterium smegma	125	63	125	63	31	125	125, 63p	250	125, 63p	125	63	63			
Pseudomonas aeruginosa	—	—	—	125	—	250	125	250	—	—	—	250			
Staphylococcus aureus	16	4	125	0.5	31	16	31	500, 250p	4	63	0.5	16			
FUNGI															
Aspergillus niger	125	125	125	125	63, 31p	125p	31	250p	125p	125	125p	16, 8p	8	>125	63
Chaetomium globosum	8	1	63	4	16	125	1	250	31	31	8	2	1	<0.25	4, 2p
Gliomastix convoluta	31	16	125	63, 31p	16	31, 16p	31	>250	16	125	63, 31p	8, 4p	4	63, 31p	16
Memnoniella echinata	4	16	125	16	8	125	4	125, 63p	125, 63p	125	16	2	0.5	0.5	16, 8p
Myrothecium verrucaria	125, 63p	63	125	63	31	125p	31, 16p	250p	125p	125	125	16, 8p	8	>125	63
Penicillium notatum	125, 63p	63, 31p	125	125	31, 16p	125	16	250p	125	125	125	8	8, 4p	>125	63
Phycomyces blakesleeanus	125, 63p	63	125, 63p	125, 63p	31, 16p	125	16, 8p	250	125	125, 63p	125	2, 1p	2	>125	63
Saccharomyces cerevisiae	63, 31p	31	>125	>250	31, 16p	>125	16	>250	>125	>125	>125	31	16	>125	>125
Stemphylium consortiale	125	63, 31p	125	125, 63p	8	125	4, 2p	250	125	125, 63p	125	16, 8p	4	>125	63, 31p
Trichophyton mentagrophytes	31	8, 4p	63	8	16, 8p	125, 63p	4	250	63	31, 16p	16, 8p	2	<0.25	0.5	8

and *B. mycoides* were grown at 30° C; the balance of the fungi were incubated at 25° C, and of the bacteria at 35–37° C. No distinction was made by our methods between the effect of the agent on fungus spore germination and on mycelial growth. Variations of pH, composition of medium, temperature and similar factors which influence toxicity were not investigated. The figures given in the table are the minimum inhibitory concentrations in micrograms per ml (48 hrs. for *M. smegma*, 24 hrs. for the other bacteria, and 42 to 48 hrs. for the fungi). They represent orders of magnitude rather than absolute values.

For the fungi, *p*-methoxy-tetrachlorophenol was as toxic or more so than tetrachloroquinone (phygon) or the dichloro-naphthaquinone (spergon), but somewhat less toxic than pentachlorophenol. Hydroquinone and *p*-methoxy-phenol were inactive in the amounts and under the conditions used. The other compounds tested were intermediate in their effects.

The importance of the test organism in evaluating toxicity is illustrated by some of the results. *Aspergillus niger*, for example, was relatively resistant to a number of the test substances, tetrachlorohydroquinone, pentabromophenol, 5-methoxy-toluquinone, to which several of the other fungi were quite sensitive. On the other hand, it was as sensitive as, or more so, to *p*-methoxy-tetrachlorophenol, penta-chlorophenol and phygon, than were some of the other fungi. *Memnoniella echinata*, *Trichophyton mentagrophytes* and *Chaetomium globosum* were inhibited by small amounts (1 ppm or less) of pentabromophenol while the other fungi were relatively insensitive to this compound (63 ppm or more).

p-Methoxy-tetrachlorophenol was less effective on the bacteria tested than on the fungi. It was less toxic for the bacteria than was 5-methoxy-toluquinone or toluquinone. Here also, toxicity varied considerably with the test organism. Generally speaking, *Staphylococcus aureus* was the most sensitive while *Escherichia coli* and *Pseudomonas aeruginosa* were the most highly resistant.

Some correlations between chemical structure and toxicity are suggested by the data in TABLE I. However, since biological activity depends not only on chemical structure, but also on the biological system (the organism) on which the chemical acts, it is not possible to generalize.

In antiphage tests carried out with *p*-methoxy-tetrachlorophenol, by Dr. I. N. Asheshov, the compound showed considerable activity against a number of staphylophages, and stimulation of a number of cholera phages.[4]

For animal toxicity tests, four groups of five mice each were used.

[4] Personal communication.

No acute toxicity was observed during a ten-day period after intravenous injection of *p*-methoxy-tetrachlorophenol (dissolved in 0.5 cc of normal saline, and adjusted to pH 7.6) in single doses up to 1 mg per mouse (ca. 50 mg per kilo).

Tested as a molluscacide, by Dr. H. W. Bond, *p*-methoxy-tetrachlorophenol gave a 100 percent kill of the snail *Australorbis glabratus* at a concentration of 10 parts per million in water, for an exposure period of 24 hours. The rate of kill, however, was less than that obtained with pentachlorophenol at the same concentration.[4]

According to Dr. G. W. McNew, *p*-methoxy-tetrachlorophenol was as effective as spergon but less effective than phygon in killing spores of *Sclerotinia fructicola* and *Alternaria oleracea* as determined by slide germination tests. Sprayed on tomato plants it burned about 10 percent of the foliage at 200 ppm, 100 percent at 400 ppm, and was ineffective in controlling early blight.[4]

p-Methoxy-tetrachlorophenol compares favorably, as an antifungal agent, with spergon and phygon. Of related phenols and quinones tested, only pentachlorophenol showed higher potency. Our results suggest that further investigation of the effect of this compound on fungi parasitic on animals might be desirable.

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FUNGI IN AIR OVER THE ATLANTIC OCEAN [1]

S. M. Pady [2] and L. Kapica [3]

(with 4 figures)

The presence of fungus spores in the air has long been known, and their relationship to epiphytotics has been fairly well established (**17**). Most of the investigations have used vaselined slides or nutrient plates exposed by hand from aircraft (**18**) or from a stationary site, and were qualitative in nature (**1**). Recently there has been considerable interest in quantitative methods as well (**3**, **4**, **5**, **6**, **16**) and considerable information has been obtained, particularly in the Canadian arctic and over other parts of Canada (**7**, **13**). Very little, however, is known of the fungi in the air over ocean masses. Meier (**10**) established that fungi were present over the Caribbean Sea, and with the cooperation of Col. C. A. Lindbergh exposed slides over the North Atlantic on which many fungus spores were caught (**8**, **9**). Newman (**11**), over the Pacific Ocean, exposed slides with a coating of agar and obtained colonies of *Cladosporium*. In 1951 two flights were made from Montreal, Canada, to London, England, and samples were taken throughout the two trips. A brief account of the numbers of fungi and bacteria has already been published (**14**). The methods, techniques, meteorological data, and a preliminary account of the fungi and bacteria obtained have also been published (**15**). This paper gives further information on the numbers and types of fungi in transatlantic air.

For the technical data on the flight reference should be made to earlier papers (**6**, **14**, **15**), but the essential features will be repeated here. The samplers used were the McGill-GE sampler, the Bourdillon slit

[1] This work was part of a cooperative project carried on at McGill University between the senior author in the Department of Botany, and Dr. C. D. Kelly in the Department of Bacteriology, and was supported by a grant (No. 175) from the Defence Research Board. The trip of June 1951 was made by Dr. C. D. Kelly, that of August by Kelly and Pady. The cooperation of Mr. G. W. Rowley of the Defence Research Board and the officers and men of Squadron 426 of the RCAF at Dorval, Quebec, and the Meteorological Office of the Department of Transport at Montreal, is gratefully acknowledged.

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sampler, and a glass wool filter (6). The samplers were installed in a North Star Aircraft (DC-6) of Squadron 426 of the RCAF, a heavy service transport. Samplers were operated continuously during the flights, which were at altitudes of 8000 and 9000 feet. Plates with modified Czapek's agar and slides coated with silicone (DC-4) were used. The McGill-GE and slit samplers were operated at an air flow of one cubic foot per minute and the sampling period was usually 15 or 30 minutes over land and 30 or 60 minutes over water.

NUMBERS OF FUNGI

Extreme variation characterized the samples taken on both the June and September flights. Not only was there variation of numbers in different air masses, but there were also variations in the samplers themselves even when sampling the same air. The slit sampler gave readings that were two to eight times higher than the McGill-GE sampler. It is not known why these two samplers, which gave comparable results when used in a fixed position, should be so dissimilar when mounted in an airplane. The numbers as obtained by the slit sampler are given in FIGS. 1–4. Each sample is represented by a single bar, solid bars indicating agar plates, and open bars silicone slides. The bars do not

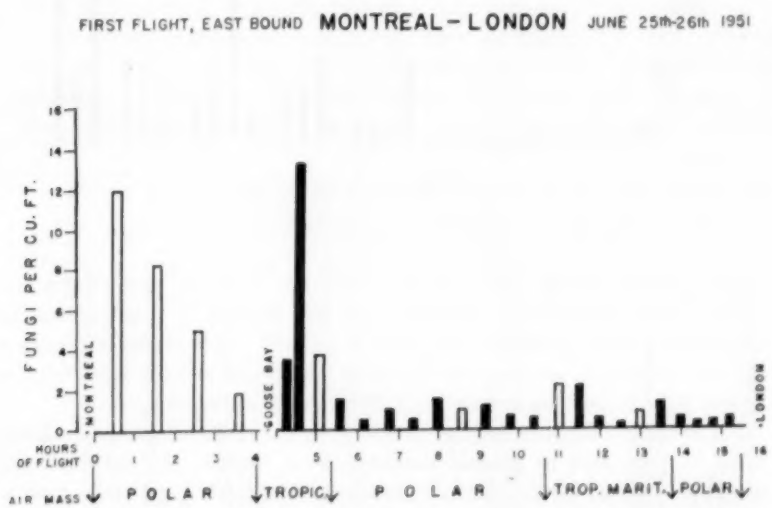

FIG. 1. Numbers of fungi in air between Montreal and London in June, 1951. Each bar represents an exposure in the slit sampler; solid bars are colonies on agar plates, clear bars are fungus spores on silicone slides. See text for details.

indicate exposure length which was usually 30 min. over the ocean and 15 min. over land. Air masses and stopovers are also included in these figures.

In the first flight (FIG. 1) on June 25, technical difficulties prevented plates from being exposed between Montreal and Goose Bay, but four hour-long samples were made with silicone slides. When the flight was resumed a mass of tropical air was encountered in which numbers of fungi in plates were high, with one plate having a reading of 13 per cubic foot. The slide exposed at this time, however, had a reading of 3.7 per cubic foot, due possibly to the fact that part of the exposure may have been in polar air. In this flight numbers over the ocean were low in all air masses and even in that which covered England. The return

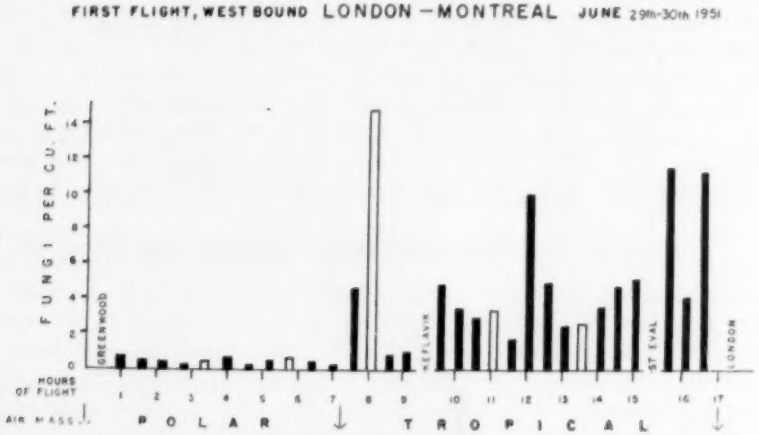

FIG. 2. Numbers on return trip from London to Montreal in June, 1951.

flight on June 29–30 (FIG. 2) was over the northern part of the Atlantic Ocean via Keflavik, Iceland, and was marked by a large mass of tropical air which extended well beyond Iceland. This air mass averaged 4.8 per cubic foot, whereas the polar air which covered the western part of the Atlantic had an average of 0.37 fungi per cubic foot.

The second flight in August (FIGS. 3, 4) encountered more air masses than in June and in general numbers were higher. In the eastward flight on August 22–23 (FIG. 3) numbers were fairly high even in polar air over land. Over the ocean, polar air with very low numbers (average 0.2 per cubic foot) was dominant, while the maritime tropical air over the eastern part of the Atlantic and over England was distinct from the polar air, with numbers averaging 1.38 per cubic foot. When the

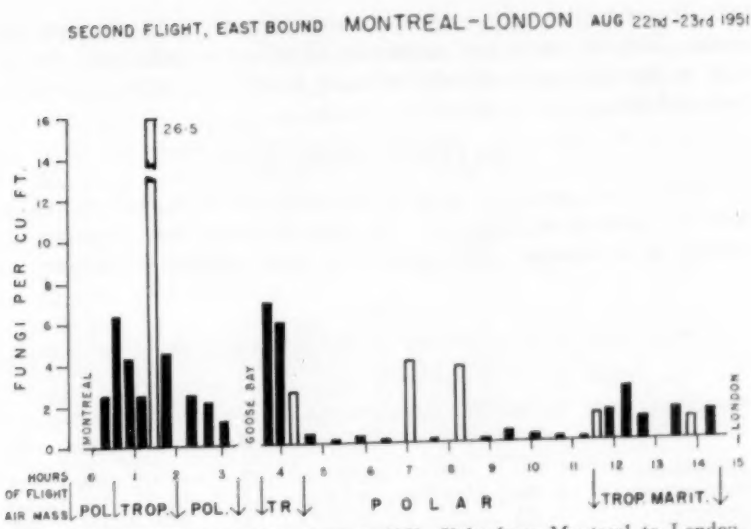

FIG. 3. Numbers of fungi in August, 1951, flight from Montreal to London.

return trip (FIG. 4) was made three days later tropical air covered England and Scotland, extending out over the ocean about 200 miles. Here the numbers of fungi were relatively high, averaging 7.3 per cubic foot. The polar air which covered the western part of the Atlantic had low numbers (average 0.5 per cubic foot), comparable with the polar air encountered over the ocean in June. Over Quebec between Goose Bay

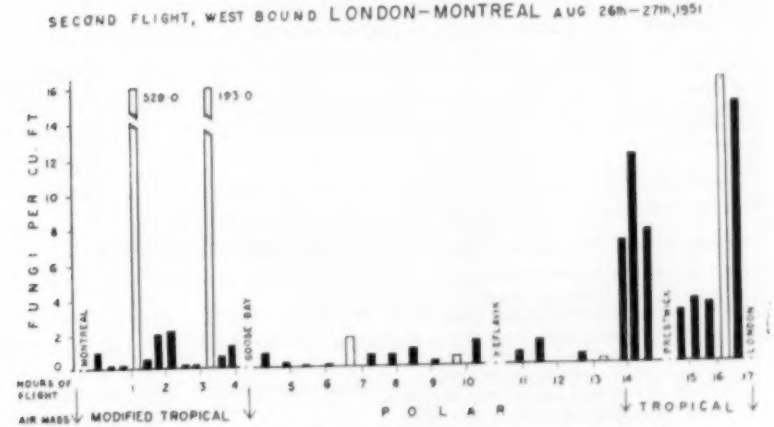

FIG. 4. Return flight from London to Montreal, August, 1951.

and Montreal, plate counts were comparable with polar air over the ocean. This air mass was tentatively identified as polar, but the results of the slide study clearly indicated a different origin, as will be discussed later.

FUNGI FROM NUTRIENT PLATES

TABLE I is a summary of all of the fungi that were obtained on the June and September flights by the three samplers, and arranged according to air masses. The plates that were exposed in tropical air

TABLE I

SUMMARY OF NUMBERS OF COLONIES FROM ALL PLATES FROM ALL SAMPLERS

Genera	Air mass						Total	
	P	P(?)mod.	m T	T mod	T(?)	T	Colonies	%
Cladosporium	596	25	196	87	285	2919+	4108	82.3
Sterile colonies	59	1	4	21	3+	72	160	3.2
Alternaria	30	1	9	4	8	82+	134	2.6
Pullularia	38	1	10	11	7	51	118	2.3
Yeasts	25	1	6		2	75	109	2.1
Penicillium	18		12	13	10	35	88	1.6
Botrytis	15	1	4	13	5	31	69	1.5
Stemphylium	20		2	2	3	25	52	1.1
Papularia	19[1]		5		2	5	31	0.6
Fusarium	10				14	4	28	0.5
Actinomycetes	5		2			17	24	0.5
Aspergillus	2		7[2]	1	1	8	19	0.4
Oospora	4			7			11	
Phoma	3		2	1	1	2	9	
Helminthosporium	1		1			2	4	
Cephalothecium	1					3	4	
Zythia	2					1	3	
Trichoderma	1					2	3	
Verticillium			1		1	1	3	
Spicaria	2					1	3	
Sporormia	2						2	1.2
Nigrospora	2						2	
Cephalosporium	1					1	2	
Sphaeronema						1	1	
Coniothyrium				1			1	
Chaetomium	1						1	
Unidentified					1	1	2	
Moss protonema	5					4	9	
Totals	857	30	261	161	343	3339+	4991	

Legend: P—Polar air mass
P mod—modified polar
T—tropical
T mod—modified tropical
m T—maritime tropical

[1] 14 of 19 colonies came from one filter.
[2] 6 of 7 colonies came from one filter.

were often overcrowded and the number reported is a minimum figure. This was particularly true of *Cladosporium*, which was the commonest fungus in the air, being present in all air masses and accounting for 82.3% of the 4991 colonies examined. Other than *Cladosporium* no one type of fungus could be said to be abundant. The order of frequency was *Alternaria* (2.6%), *Pullularia* (2.3), yeasts (2.1), *Penicillium* (1.6), *Botrytis* (1.5), and *Stemphylium* (1.1) with other fungi occurring in less than 1% of the total (TABLE I). Nonsporulating colonies were second in order of frequency (3.2%) but their magnitude was much lower than in the arctic (12).

In TABLE II an attempt has been made to indicate the genera of fungi that were obtained in the plates, on a cubic foot basis, and averaged for the respective air masses. The data from the filters have not been included in this table. Quantitative calculations were made for *Cladosporium*, *Alternaria*, *Stemphylium*, *Penicillium*, *Aspergillus*, Actinomycetes, yeasts, *Pullularia*, and *Fusarium*. The remaining genera, referred to in TABLE I, are grouped under the heading "Other Ident." and nonsporulating sterile colonies are also included. In this table the discrepancies in the readings of the McGILL-GE and slit samplers are clearly demonstrated.

Quantitatively, *Cladosporium* ranged from 0.01 to 0.1 per cubic foot with the McGill-GE sampler in polar air with a high of 1.01 per cubic foot in an air mass that was either modified polar or tropical air. With the slit sampler, however, *Cladosporium* in polar air had a range of 0.14 to 2.4 per cubic foot (the latter reading being over land), with the unidentified air mass referred to above having 3.9 per cubic foot. In tropical air over the western Atlantic a concentration of 7.8 per cubic foot was obtained, whereas over midocean, which was usually dominated by polar air, *Cladosporium* did not exceed 0.8 per cubic foot. *Cladosporium herbarum* Link ex Fr. was the commonest species: *C. cladosporioides* (Fres.) deVries and *C. macrocarpum* Preuss were found occasionally.[4]

Alternaria was found in all air masses, with the highest reading being 0.52 per cubic foot in tropical air (TABLE II). *Pullularia pullulans* (de Bary) Berkh. was also present in all air masses with a maximum concentration of 0.36 per cubic foot in one tropical air mass in mid-Atlantic. Yeasts were identified in all but one air mass and were highest (0.26 per cubic foot) in the same air mass as *Pullularia*. The fungi

[4] The species of *Cladosporium* were kindly identified by Dr. G. A. de Vries, Centraalbureau, Baarn.

TABLE II

NUMBERS OF FUNGI PER CUBIC FOOT FROM COLONIES IN PLATES EXPOSED IN THE McGILL-GE AND SLIT SAMPLERS IN TWO TRANSATLANTIC TRIPS IN JUNE AND AUGUST, 1951. SEE TEXT FOR DETAILS.

				Plate fungi per cubic feet (GE and Slit)											
Trip	Air mass	Sample	Method	*Clad.*	*Alt.*	*Stem.*	*Pen.*	*Asp.*	Act.	Yeast	*Pull.*	*Fus.*	Other ident.	Sterile	Total
Montreal to London	Polar, 4 hrs.														
	Tropical 1° 30" (Labrador & W. Atlantic)	6	GE	1.7	0.0	0.0	0.0	0.03	0.0	0.0	0.03	0.0	0.0	0.1	1.9
			Sl	7.8	0.1	0.03	0.03	0.0	0.03	0.03	0.0	0.0	0.0	0.1	8.2
	Polar 5° (mid Atlantic)	21	GE	0.07	0.0	0.0	0.0	0.0	0.0	0.0	0.01	0.01	0.0	0.006	0.1
			Sl	0.88	0.01	0.003	0.01	0.0	0.0	0.003	0.01	0.0	0.01	0.015	0.93
	Trop.-Maritime 3° (mid & E. Atl.)	13	GE	0.09	0.0	0.0	0.0	0.0	0.0	0.03	0.02	0.0	0.0	0.01	0.17
			Sl	1.0	0.01	0.01	0.01	0.0	0.0	0.01	0.01	0.0	0.02	0.01	1.1
	Mod. polar (?) 1° 30" (E. Atlantic)	7	GE	0.1	0.0	0.0	0.0	0.0	0.0	0.02	0.0	0.0	0.0	0.0	0.12
			Sl	0.37	0.01	0.0	0.0	0.0	0.0	0.0	0.01	0.0	0.01	0.01	0.4
London to Montreal	Tropical 9° 45" (to 600 mi. W. Iceland)	42	GE	1.44	0.005	0.005	0.02	0.0	0.0	0.07	0.01	0.0	0.01	0.1	1.72
			Sl	4.42	0.02	0.02	0.02	0.0	0.04	0.09	0.04	0.0	0.05	0.05	4.82
	Polar 7° 15" (W. Atlantic)	24	GE	0.10	0.003	0.0	0.006	0.0	0.0	0.04	0.0	0.0	0.0	0.04	0.14
			Sl	0.28	0.003	0.01	0.003	0.01	0.0	0.03	0.003	0.0	0.02	0.02	0.37

TABLE II—*Continued*

Plate fungi per cubic feet (GE and Slit)

Trip	Air mass	Sample	Method	*Clad.*	*Alt.*	*Stem.*	*Pen.*	*Asp.*	Act.	Yeast	*Pull.*	*Fus.*	Other ident.	Sterile	Total
Montreal to London	Polar 30′′ (over Labrador)	2	GE	0.8	0.1	0.0	0.1	0.0	0.0	0.27	0.1	0.0	0.0	0.0	1.5
			Sl	2.4	0.0	0.0	0.0	0.0	0.0	0.0	0.08	0.0	0.08	0.0	2.6
	Trop. or mod. polar 1° 30′′ (Labrador)	11	GE	1.01	0.0	0.0	0.1	0.01	0.0	0.03	0.0	0.32	0.1	0.01	1.6
			Sl	3.9	0.13	0.05	0.08	0.0	0.0	0.0	0.13	0.0	0.06	0.10	4.5
	Polar 1° 30′′ (Labrador)	6	GE	0.3	0.0	0.03	0.03	0.0	0.03	0.03	0.0	0.17	0.0	0.03	0.6
			Sl	1.6	0.09	0.09	0.02	0.0	0.0	0.04	0.06	0.02	0.09	0.0	2.0
	Tropical 1° (E. Atlantic)	7	GE	0.63	0.0	0.0	0.04	0.0	0.04	0.0	0.04	0.09	0.09	0.0	0.93
			Sl	4.65	0.46	0.06	0.20	0.0	0.0	0.26	0.36	0.0	0.13	0.06	6.45
	Polar 7° (mid Atlantic)	26	GE	0.01	0.02	0.0	0.0	0.0	0.005	0.0	0.0	0.01	0.0	0.0	0.05
			Sl	0.14	0.01	0.01	0.006	0.0	0.003	0.01	0.003	0.0	0.03	0.006	0.20
	Trop. Marit. 3° (E. Atlantic)	16	GE	0.11	0.0	0.0	0.03	0.0	0.015	0.0	0.0	0.0	0.01	0.0	0.17
			Sl	0.92	0.07	0.01	0.08	0.01	0.01	0.01	0.08	0.0	0.07	0.01	1.38
London to Montreal	Tropical 2° 45′′ (to 200 mi. W. Scotland, Eng., & E. Atlantic)	17	GE	0.72	0.07	0.02	0.03	0.05	0.04	0.0	0.04	0.0	0.09	0.11	1.34
			Sl	5.89	0.52	0.12	0.03	0.01	0.03	0.03	0.19	0.01	0.12	0.18	7.3
	Polar 9° 45′′ (mid & W. Atl.)	31	GE	0.05	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.01	0.03	0.12
			Sl	0.36	0.02	0.03	0.01	0.0	0.0	0.01	0.03	0.0	0.03	0.03	0.54
	Mod. Trop. 3° 50′′ (over E. Canada)	23	GE	0.19	0.0	0.0	0.08	0.01	0.0	0.0	0.0	0.0	0.11	0.13	0.46
			Sl	0.46	0.02	0.01	0.03	0.0	0.0	0.0	0.07	0.0	0.08	0.07	0.87

Stemphylium, *Penicillium*, *Aspergillus*, Actinomycetes (*Streptomyces*), and *Fusarium* are characterized by low concentrations and rather general distribution in the air masses that were encountered. In addition to the above, the following fungi were occasionally present but in insufficient numbers to calculate on a cubic foot basis: *Botrytis*, *Papularia*, *Oospora*, *Phoma*, *Helminthosporium*, *Cephalothecium*, *Zythia*, *Trichoderma*, *Verticillium*, *Spicaria*, *Sporormia*, *Nigrospora*, *Cephalosporium*, *Sphaeronema*, *Coniothyrium*, and *Chaetomium*.

There was very little indication of correlation between genera and air masses. *Cladosporium*, for example, constituted 69.5 and 83.3% of the polar air and 75.0, 54.0, 83.0 and 87.4% of tropical air masses (TABLE I). *Alternaria*, yeasts, *Botrytis* and *Penicillium* were more abundant in tropical air than in polar air: these genera constituted 19.5 and 11.5%, 17.7 and 9.5%, 7.4 and 5.7%, and 8.3 and 6.9% in tropical and polar air masses, respectively. On the other hand, *Stemphylium*, *Pullularia*, *Fusarium* and *Papularia* were more numerous in polar air, constituting 14.5 and 12.0%, 3.8 and 0.9%, and 7.2 and 1.2% of the respective polar and tropical air masses. *Sporormia* was found only in polar air, confirming previous observations concerning its northern range and arctic habitat (**12**).

SILICONE SLIDE STUDY

Because previous work (**13**) had indicated that large numbers of fungus spores were sometimes present in the atmosphere, silicone slides were periodically exposed in the slit sampler (TABLE III). The number of spores per cubic foot varied from 0.2 in polar air over the ocean to over 500 in modified tropical air over eastern Canada. Over the ocean, polar air had much lower numbers than tropical air; for example, between Keflavik, Iceland, and Greenwood, Nova Scotia, over the western Atlantic, one slide was exposed in tropical air, and two in polar air, giving readings of 15.1, 0.56 and 0.4 per cubic foot, respectively.

Cladosporium spores were the commonest type of spore on the slides, as might be expected from the plate counts (TABLE I), appearing on all but 2 slides and constituting as much as 80% of the total. Over the ocean, numbers varied from 0.7 per cubic foot in polar air to 9.0 per cubic foot in tropical air. The spores were commonly in clumps or groups. On one slide exposed over the western part of the Atlantic, there were groups of 18, 7, 4, 2, 2, all within a small area on the slide. *Alternaria* spores were found on 17 of 25 slides, with a high of 1.8 per cubic foot in tropical air over England; in polar air they were either absent or low in numbers, not exceeding 0.1 per cubic foot.

TABLE III

FUNGUS SPORES, HYPHAE AND POLLEN PER CU. FT. FROM SILICONE SLIDES EXPOSED IN THE SLIT SAMPLER

	Air mass	*Clad.*	*Alt.*	Smuts	Hyphae	Yeasts	Pollen	Total per cu. ft.
Montreal–Goose Bay	P(?)	7.7	0.4	0.6	0.2	0.4	0.5	12.1
	P(?)	6.5	0.2	0.3	0.1		0.3	8.2
	P(?)	3.1	0.2	0.2	0.03	0.05	0.8	4.9
	P(?)	1.4	0.04	0.04			0.1	1.8
Goose Bay–London	T	3.1	0.3				0.25	3.7
	P	0.75	0.1				0.02	1.0
	m T	1.4	0.3	0.04	0.04		0.1	2.2
	T	0.4		0.2				0.8
St. Eval–Keflavik	T	2.1		0.4	0.05		1.1	2.6
	T	2.1	0.1	1.0			0.9	3.4
Keflavik–Greenwood	T	9.0	0.4	4.5	0.2		0.7	15.1
	P	0.23		0.02		0.13	0.1	0.56
	P	0.3				0.05	0.16	0.4
Montreal–Goose Bay	T(?)	7.6	0.64	2.7	1.36	5.0	0.56	26.5
Goose Bay–London	T	0.6	0.2	0.4	0.06	0.06	0.6	2.46
	P	0.26	0.03	0.03		0.8		4.1
	P	0.4				1.1	0.4	3.8
	m T	0.53					0.06	1.26
	m T	0.5		0.4			0.3	1.1
London–Prestwick	T	8.3	1.8	0.4	0.9		4.5	16.3
Prestwick–Keflavik	P							0.2
Keflavik–Goose Bay	P		0.05				0.06	0.35
	P	0.6	0.06	0.2			0.6	1.6
Goose Bay–Montreal	T mod.	2.8	0.8	1.4	0.5	4.5±	1.6	193.8
	T mod.	4.2	0.1	0.1		282.0±	0.8*	529.0

* Moss only

Legend: P—polar air mass
T—tropical
T mod.—modified tropical
m T—maritime tropical

Smut spores were present on all slides exposed over land, and on 9 of the 16 exposed over the ocean. In tropical air, spores were present on all but two slides, reaching a concentration of 4.5 per cubic foot on June 29, west of Iceland. Polar air, on the other hand, had low numbers, 5 of 8 slides being negative, and two slides containing but a single spore. The *Ustilago* spores were of the *U. hordei* type. *Tilletia* spores were found on only two slides, exposed over eastern Canada in August. Rust spores were comparatively rare, occurring as single spores on 5

slides, 4 of which were over eastern Canada, and one spore in the same tropical air mass in which smut spores were most numerous. Yeast cells were present on only 10 of the slides and were irregular in their occurrence in tropical and polar air masses. In general their numbers were low over the ocean even in tropical air, while over land a concentration of 4.5 per cubic foot was obtained over England in August.

Spores of the following genera were found infrequently over the ocean and were not calculated quantitatively: *Stemphylium* on 14 slides, 6 over land; *Fusarium* 12, 4 over land; *Septoria* 3, 2 over land; *Sporormia* 2; *Helminthosporium* 2; *Leptosphaeria* 1; *Pleospora* 1, over land. *Penicillium* and *Aspergillus* spores were not generally recognized on the slides but their presence in the air is indicated by the colonies which developed in the plates (TABLE I). *Dematium*-like chains of spores were occasionally observed.

The commonest spores on the slides were single-celled, round to oval, hyaline or colored and were not identifiable as to genus. This was particularly true of the air over eastern Canada on August 26, which had high numbers of single celled spores, of which yeasts constituted 282 + per cubic foot and yellow-brown spores 211 per cubic foot. About 50% of the latter had an apiculus and were considered to be basidiospores.

Hyphal fragments were present on 10 of the slides and were abundant in tropical air in concentrations up to 1.36 per cubic foot, but were almost completely absent in polar air. Most of the fragments were dematiaceous and were frequently conidiophores of the *Alternaria* type. Terminal sections of conidiophores were common and occasionally the entire conidiophore was present. In a few cases an intact cluster or clump of conidiophores was observed (TABLE III).

Pollen grains were present on 20 of the slides (TABLE III) and were most abundant over land, the highest concentration being 4.5 per cubic foot over England in August. Over the ocean, numbers did not exceed 0.7 per cubic foot even in tropical air. Some moss spores were obtained over eastern Canada in August and these have been included with the pollen grains.

COMPARISON OF PLATE AND SLIDE READINGS

In TABLE IV a comparison has been made of the numbers of fungi obtained on silicone slides and the numbers as determined by colony formation on plates exposed at the same time. In all cases the slides gave readings which were many times higher than the plates. There are several factors involved in this comparison: the slit sampler in which

the slides were exposed gives higher readings than the McGill-GE sampler in which the plates were exposed, as has been pointed out earlier; fungus spores were often present in the air in groups, all the individuals being counted on the slide, whereas on the plate a single colony would probably develop; many of the fungus spores were either dead or were unable to grow on the medium used. *Cladosporium*, *Alternaria* and yeasts were chosen for comparison as they grow well on our medium and can be identified on the slides. All three of these fungi were more numerous on the slides than in the plates. *Cladosporium*

TABLE IV

LOSS OF VIABILITY BY COMPARISON OF FUNGUS SPORES ON SILICONE SLIDES (SL) EXPOSED IN THE SLIT SAMPLER AND COLONIES PRODUCED BY SIMULTANEOUS EXPOSURE OF PLATES (PL) IN THE MCGILL-GE SAMPLER

	Air mass	Total		*Cladosporium*		*Alternaria*		Yeasts	
		Sl	Pl	Sl	Pl	Sl	Sl	Sl	Pl
Goose Bay–London, June 25	T	3.7	0.2	3.1	0.06	0.3			
	P	1.0	0.05	0.8	0.05	0.1			
Keflavik–Greenwood, June 29	T	15.1	1.9	9.0	1.56	0.4			0.13[1]
	P	0.56		0.23				0.13	
Montreal–Goose Bay, Aug. 22	T(?)	26.5	1.7	7.6	1.2	0.6		5.0	
Goose Bay–London, Aug. 22	P	3.8	0.1	0.4	0.06			1.1	
	mT	1.3		0.5					
London–Prestwick, Aug. 29	T	16.3	0.4	8.3	0.26	1.8	0.13		
Keflavik–Goose Bay, Aug. 29	P	1.6	0.1	0.6		0.06			
Goose Bay–Montreal, Aug. 29	T mod.	529.0+	0.9	4.2	0.4	0.1		282.0+	

[1] Smuts were 4.5 per cu. ft. on the slide and these may be smut colonies.

Legend: P—polar air mass
T—tropical
T. mod.—modified tropical
m T—maritime tropical

illustrates this well, as it was present on all but two slides up to 9.0 per cubic foot, whereas the highest concentration in the plates was 1.56 per cubic foot, and three plates had no *Cladosporium* colonies whatsoever. *Alternaria* and yeast were similar except that they were lower in concentration and colonies were present in but a single plate. TABLE IV shows that there is a definite loss in viability of fungus spores while air borne and also that some spores are able to survive a transatlantic crossing in a viable condition.

Polar air usually had low numbers of fungi both on the plates (TABLE II) and slides (TABLE III). Tropical air, on the other hand, usually had high numbers of spores, but over the ocean only a few were able to

develop into colonies (TABLE IV, lines 3, 8). Maritime tropical air (TABLE IV, line 7) had much lower numbers, due to its origin over water. The air mass which covered eastern Canada on August 29, had a load of fungus spores (TABLE IV, bottom line) that was higher than any exposed in any previous aerobiological work, yet the colony counts were very low and yeasts which constituted over half of the spores were not represented by a single colony on the plates. This air mass had been identified by the meteorologists as polar, because of its southerly flow and other characteristics. The plate count seemed to confirm this identification also. Later when the slides were read and the remarkably high spore load was discovered, the identification was revised and it was considered to be tropical air of continental origin that had passed into the Arctic and was now moving southeast. The failure of the yeasts to form colonies indicates that they had been in the air a long time and had lost their viability. The value of the slides is clearly illustrated here since it revealed an unexpectedly high load of fungus spores and established this air mass as tropical in origin rather than polar.

DISCUSSION OF RESULTS

There was no evidence of gradual diminution of organisms in the air over the ocean as distance from land increased. The data from the two transatlantic flights reported here indicate rather that the number of fungi in the air over any given part of the Atlantic Ocean depends not upon its proximity to or distance from land but upon the kind of air mass and the length of time the fungi have been in the air. The agricultural lands of North America appear to be the chief source of air borne fungi (**12**) and tropical air which passes over them carries the highest loads. When such air masses move northward, they may have lost much of their spore load by precipitation but may still carry substantial numbers of organisms, particularly fungus spores. Pady and Kelly (**13**) found that in the Arctic a tropical air mass over Hudson Bay had a fungus spore load as high as 78.0 per cubic foot, of which approximately one per cubic foot was viable, while in polar air north of it, fungus spores were 1.17 per cubic foot, and the viable fungi in the plates varied from 0.01 to 0.4 per cubic foot.

Because westerly winds are dominant over the Atlantic Ocean, it is not unusual for air masses which have reached arctic regions to move eastward out over the ocean. Samples taken in this air would be expected to be somewhat similar to those taken in the Arctic but with somewhat lower numbers. The data presented here provide support for

this hypothesis, tropical air having fairly high numbers of fungus spores, with considerable loss in viability and polar air with low numbers of spores and correspondingly low viability. The spore load correlates well with continental air masses and may be very helpful in identifying air masses, such as the one that covered eastern Canada on August 29, which was at first identified as polar because of northerly air flow and low plate counts, but when the high spore load was discovered was changed to modified tropical. Plate counts in such air masses thus do not reflect the origin but do provide valuable data on the ability of fungus spores to survive prolonged exposure in the air.

The presence of viable fungi over the Atlantic Ocean confirms the observations of Meier (**10**) over the Caribbean Sea, and Newman (**11**) over the Pacific Ocean. The latter found viable spores of *Cladosporium herbarum* and a few other fungi, while on the slides, spores of *Fungi Imperfecti* and rusts were present, as well as hyphal fragments. The flora over the Atlantic would appear to be similar to that of the Pacific Ocean except that rusts were rare, one spore only being found, whereas smuts were present on 12 of the 16 slides exposed over the ocean, and in some tropical air masses in considerable abundance (TABLE III). It is not known whether these chlamydospores were viable or not, but the presence in one air mass of yeastlike colonies (TABLE IV) where smuts were 4.5 per cubic foot and yeasts were absent on the slides, suggests that these may have been smut colonies.

It is not known whether the air masses over the ocean have a characteristic flora. The presence of *Cladosporium herbarum* as the main constituent of both tropical and polar air over the ocean suggests that the differences are chiefly quantitative. Unless the arctic regions have a large number of indigenous fungi, the air masses that lie over the northern part of North America would be essentially tropical air with fungi picked up from the Great Plains region and modified by trajectory and meteorological changes. Pady and Kelly (**13**) found that most of the fungi in arctic air were soil organisms, and it was presumed that they had been carried northward from agricultural regions. The fungi obtained over the ocean were chiefly soil fungi and they undoubtedly had a similar origin.

There is probably a correlation between the length of time in the air and viability. Not all fungi grow uniformly on all media, and the medium used in this work may have been highly selective. *Cladosporium* provides a valuable tool in this connection since it can be recognized readily on the silicone slides and also grows well on the medium. In both tropical and polar air there were much higher numbers of

Cladosporium as spores on slides than as colonies in the agar plates, which indicates that many of the spores were no longer viable. This would suggest that there is a time factor involved and that a significant viability ratio could be worked out. For example, in *Cladosporium* the ratio between total spores and viable spores as determined by the formation of colonies in the tropical air mass over eastern Canada on August 29, is 11 : 1, while in the tropical air over the eastern Atlantic on the same trip, the ratio was 31 : 1. On the other hand, *Alternaria* was present on 7 of the 10 slides in the 10 air masses selected in TABLE IV but in only one air mass were there any viable spores and that over England in August where the viability ratio was 14 : 1. It would be desirable to know if there are characteristic viability ratios for the air-born fungi.

The presence of sterile nonsporulating colonies was a characteristic feature of the fungi obtained in arctic air (12) and appears to be also for those obtained in transatlantic air (TABLE I). These colonies make good growth but fail to produce conidia or any other reproductive structures. Even *Cladosporium* appears to be affected, as there are many colonies of what appears to be typical *Cladosporium* but without conidia. It would be desirable to determine what factor or factors are responsible for this failure to sporulate.

Stakman (17) has pointed out that there is very little evidence regarding effective dissemination across ocean barriers and that there is no evidence of effective interchange of inoculum across the Atlantic between Europe and North America. The dominant westerly flow of air over the northern part of North America and the Atlantic Ocean would effectively prevent such an interchange of inoculum. It would not, however, prevent a successful west to east passage and the evidence presented here indicates that fungus spores successfully make these one way crossings.

SUMMARY

Exposures were made over the Atlantic Ocean on two transatlantic flights in June and August, 1951, from Montreal to London and return, using the McGill-GE, slit, and filter samplers. Nutrient plates were exposed in the McGill-GE sampler and plates and silicone slides in the slit sampler. Dilution plates were made from the filters.

Cladosporium was the commonest fungus, comprising 4108 (82.3%) of the total. Nonsporulating, *Alternaria*, *Pullularia*, yeasts, *Penicillium*, *Botrytis*, and *Stemphylium* constituted 3.2, 2.6, 2.3, 2.1, 1.6, 1.5 and 1.1% of the total colonies. The following eighteen additional genera

were present: *Papularia, Fusarium, Actinomycetes* (*Streptomyces*), *Aspergillus, Oospora, Phoma, Helminthosporium, Cephalothecium, Zythia, Trichoderma, Verticillium, Spicaria, Sporormia, Nigrospora, Cephalosporium, Sphaeronema, Coniothyrium,* and *Chaetomium.*

Quantitatively the fungi were determined on a cubic foot basis. *Cladosporium* in polar air ranged from 0.01 to 0.1 per cubic foot with the McGill-GE sampler and 0.14 to 2.4 per cubic foot with the slit sampler; in tropical air numbers were higher, reaching 7.8 per cubic foot in one air mass over the western Atlantic. *Cladosporium herbarum* was the commonest species. *C. cladosporioides* and *C. macrocarpum* were found occasionally. The remaining genera were less than one per cubic foot.

Fungus spores were determined from silicone slides exposed in the slit sampler. The number varied from 0.2 per cubic foot in polar air to 529 per cubic foot in modified tropical air. *Cladosporium* spores were most abundant, having concentrations up to 9.0 per cubic foot. In general, the same fungi were obtained on the slides as on the plates except for smuts. Chlamydospores of the *Ustilago* type were found on all but 7 slides with a high in 4.5 per cubic foot in tropical air near Iceland. Many of the fungus spores were in clusters or groups. Hyphal fragments, and often conidiophores, were present on most of the slides exposed in tropical air.

The evidence indicates no gradual diminution as distance from land increases, but that numbers in the air are correlated with air masses and fungi can successfully make the west to east crossing of the Atlantic.

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ENDOGONE IN CANADIAN RODENTS [1]

ELEANOR SILVER DOWDING [2]

(WITH MAP AND 7 FIGURES)

During a visit to the Farlow Herbarium in the spring of 1952, the late Dr. Lawrence White showed the author a slide that had been sent to him by Dr. William Jellison of the Public Health Service at the Rocky Mountain Laboratory in Montana. It was a preparation of stomach contents of a mouse. The slide contained one or two large, golden-brown, thick-walled, stalked spheres measuring up to 120 μ. Dr. White identified these bodies as spores of the Phycomycete, *Endogone*. He fetched from his herbarium yellow biscuit-shaped *Endogone* sporocarps about 2 cm across, which had been collected from beneath the soil. Presumably the mouse, when it was trapped, had been eating similar micro-truffles.

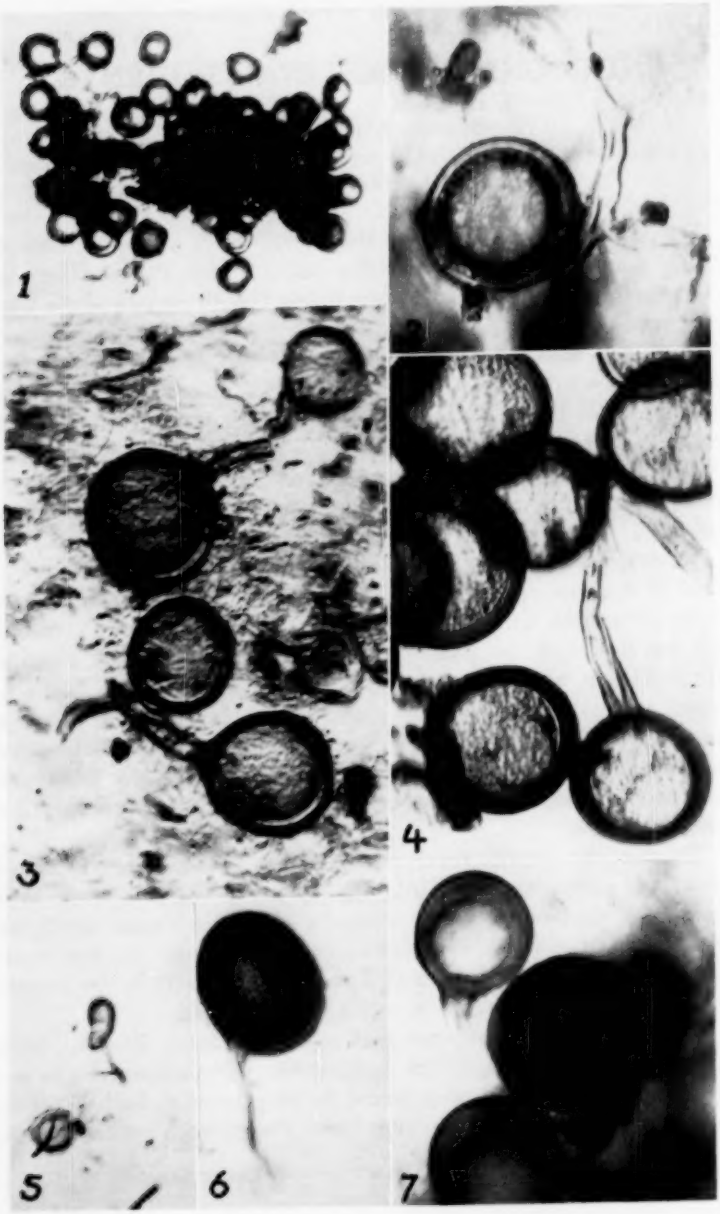
Thaxter's monograph (**5**) describes thirty species of *Endogone* collected in the New and the Old World. These had been discovered on sphagnum fronds, under leaf-cover, and in soil. Mosse (**3**), in England, has described and photographed *Endogone* sporocarps, connected with mycelium which entered into the roots of strawberry plants and formed a mycorrhizal relationship with them. It would seem that just also absorb nourishment from plant roots, in their sporulation form truffles in which they produce their spores, so certain lower fungi which also absorb nourishment from plant roots, in their sporulation form *Endogone* fruits.

Thaxter (**5**) records *Endogone* fragments in the digestive tracts of shrews and millipedes. Diehl (**1**) reports that in 1926 Mr. T. E. White found small black objects in the stomachs of mice in Kansas which were identified by Miss U. K. Charles as *Endogone* spores. He also reports that in 1939 Dr. W. J. Hamilton found similar objects in the stomachs of shrews, mice, lemmings and moles in New York.

Dr. Wm. Jellison tells me that when he visited Beaver, Alaska, recently, he found a few spores in a mouse, *Microtus oeconomus macfarlandi*. Although they were found in lung material, he believes that they were dragged over the lung from the stomach contents during

[1] This work was made possible by a Provincial Health Grant.

[2] Mrs. E. S. Keeping.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autopsy. These are the spores mentioned above which Dr. W. L. White of Harvard identified as *Endogone*.

Later, Miss Betty Locker, of Dr. Jellison's department, in a personal communication states that she found masses of similar spores in the caecum of mice, *Peromycus maniculatus*, and chipmunks, *Eutamias* sp., from the trap line of Mr. Lowell Adams, Biologist, U. S. Forest Service, Lincoln Co., Montana, and that when Dr. W. W. Diehl examined these he pronounced them *Endogone* spores.

Since small animals in Kansas, New York, Montana and Alaska eat *Endogone*, the question arises whether or not Canadian rodents have a similar diet.

SPOROCARP FRAGMENTS IN ALBERTA MICE

In July and August, 1953, under the direction of Mr. J. H. Brown, the Field Survey of the Provincial Division of Entomology collected eleven specimens of deer-mice, *Peromyscus maniculatus*. One animal was trapped in an area 50 miles west of Edmonton, two were from 150 miles in the opposite direction, and the remaining eight were from 200 miles southeast of the city. The stomachs of every one of the eleven mice contained masses of black grains about 0.5 mm in diameter (FIG. 1). Examination proved them to be fragments of sporocarps of *Endogone fasciculata* Thaxter.

Dr. J. E. Moore of the Department of Zoology then drew my attention to a fungus in the stomach contents of two grasshopper mice, *Onychomys leucogaster*, trapped near Taber, about 300 miles south of Edmonton. The same fungus was observed in these animals.

Dr. Moore then submitted for examination the stomachs of 35 small animals that he had collected throughout Alberta from areas shown on the map. The animals were of various species, including grasshopper mice, jumping mice, voles, lemmings, ground squirrels and rock rabbits. The stomachs of five of these contained *Endogone* spores. *E. fasciculata* was found in a rare vole, *Lemmiscus curtatus*, collected from the southeast corner of Alberta and in three rock rabbits, *Ochotona princeps*, collected from the southern Rocky Mountains. *E. pulvinata* Henn. was found in the stomach of a Rocky Mt. jumping mouse, *Zapus princeps*, from a mountain near Banff.

Endogone spores found in Alberta. 1-6, *E. fasciculata*; 7, *E. pulvinata*. 2, from straw; all others from rodent stomachs. 1, 3 and 4, from deer-mice; 5 and 6, from grasshopper mice; 7, from a Rocky Mountain jumping mouse. 5, upper right, zygospore; all other spores are chlamydospores. Magnification: 1 and 5, 75; all others, 200.

Professor E. H. Strickland of this University examined stomachs of grasshopper mice that contained *Endogone* spores. He found that more than half the contents were insect parts, a particularly favored diet being carabid beetles. It is possible that these beetles are mycophagists.

In Alberta, squirrels collect pilei of Russulas and wedge them in the branches of trees. Mice and squirrels are known to store and feed on fungi. Professor R. F. Shaner, of the University, in the autumn of 1953, observed four-foot high piles of *Lactarius* fruits probably collected by squirrels. Similarly, burrowing rodents, when they come across the minute *Endogone* sporocarps, may hoard them for food.

THE SPORES OF ENDOGONE FASCICULATA

The identification of *Endogone fasciculata* was based partly on finding clusters of chlamydospores, together with zygospores, in the soral fragments (FIG. 5). *E. fasciculata* is the only species except *E. sphagnophila* Atk. with both types of spores on the same sorus.

The grains of this species from Alberta mouse stomachs are composed of loose tufts of coarse brown branching hyphae very sparingly septate, bearing masses of spores in grape-like clusters (FIG. 1).

The chlamydospores are spherical or slightly elongate, more variable in diameter (35–100 μ) than Thaxter's spores (60–85 μ). The spore wall reaches the remarkable thickness of 10 μ and is composed of a thin exospore and a thick amber-colored striated endospore (FIG. 4).

In an animal's digestive tract, individual chlamydospores are frequently torn from the sorus. Such spores are stalked. They appear, as one observer described them, "like balloons on a stick" (FIG. 3). Examination of the spore and its sporophore shows why the two remain connected. At the point of attachment the wall is so thick that it almost closes the cell lumen, leaving only a narrow isthmus. The wall thickening continues down the sporophore for 30 to 180 μ, gradually tapering to the normal thickness of the hyphal walls, at which point it breaks (FIG. 4). One or two chlamydospores show lines of abscission at their bases, and some spores have broken off along this line so that they are not stalked. Such spores may have been becoming ripe and ready to be disseminated naturally at the time they were eaten.

The zygospores are irregularly elliptic (FIG. 5), "potato shaped," measuring about 84 $\times$ 15 μ, considerably larger than Thaxter's, which he himself describes as being immature.

Endogone fasciculata is the second of two fungi discovered in Al-

berta deer-mice, the first being the pulmonary *Haplosporangium parvum* (2). The pearl-like chlamydospores of *H. parvum* resemble the brown chlamydospores of *E. fasciculata* in their size, shape and wall thickness.

THE SPORES OF ENDOGONE PULVINATA

Grape-like clusters of spores from the stomach of a Rocky Mountain jumping mouse were identified as *Endogone pulvinata* Henn. because they resemble Thaxter's description of the Venezuelan type material. The chlamydospores, shown in FIG. 7, are smaller than those of *E. fasciculata,* averaging $50 \times 60\ \mu$, and the walls are thinner, only $2–4\ \mu$ thick. The walls are composed of a dark exfoliating exospore and a clear endospore. Unlike *E. fasciculata,* all the spores are separated from the hyphae by septa.

GERMINATION EXPERIMENTS

When fresh caecal material containing *Endogone fasciculata* spores is smeared on agar, species of *Mortierella* and of *Mucor* usually appear within a day or two. This is of interest because of the phylogenetic affinity between *Endogone* and these genera (**3, 6**). No evidence could be found, however, that either of these molds actually originated from the *Endogone* spores.

Chlamydospores of *Endogone* from the stomach were planted in Van Tieghem cells in malt, blood, Sabouraud's, Littman's and other agars. Before incubation, some of them were frozen at $-20°$ C for 48 hours, and others were heated for an hour in a water bath at 56° C. Some cultures were incubated at room temperature, others at 37° C, some in darkness and others in light. No chlamydospore was observed to germinate. It is possible that they had been eaten by the mice before they were mature and that, could the sporocarps themselves be collected, they might be induced to grow.

THE SEARCH FOR SPOROCARPS IN NATURE

We have evidence that in July and early August of 1953, Alberta deer-mice and other rodents over all the southern half of Alberta ate large quantities of *Endogone* (map). The writer knows of no record, however, of *Endogone* collected in Western Canada. During the latter part of August a search was made near Edmonton in sphagnum bogs, woods, and fields for sporocarps. Soil, fallen leaves, stems and fruits were examined, all unsuccessfully.

The distribution of *Endogone* in Alberta rodents

In September my assistant, Miss Arline McMicking, gathered handfuls of straw from a field just outside the laboratory. After washing it in water she examined the sediment. The first day she discovered two thick-walled spores each measuring 60 μ and on the following day, from straw from the same area, she found two more. That these spores are those of *Endogone fasciculata* can be seen from an examination of FIG. 2. It is obvious, then, that the fungus must be a common one in Alberta.

Since the field is in parkland country twenty miles away from any muskeg, the spores on the straw could scarcely have come from fruit-

bodies growing on sphagnum. It is more likely that they came from hypogeal fruits which were overlooked because they were mixed with and indistinguishable from the soil itself.

SUMMARY

In midsummer, masses of *Endogone* spores appearing as black grains to the naked eye were found in the stomachs of Alberta rodents from areas extending from Edmonton south to the United States border and from the Rocky Mountains east to the province of Saskatchewan.

E. fasciculata was found in deer-mice, rock rabbits, a grasshopper mouse and a vole; *E. pulvinata* was found in a Rocky Mountain jumping mouse.

In September, spores of *E. fasciculata* were recovered from straw gathered in a field near the laboratory.

ACKNOWLEDGMENT

The author expresses her indebtedness to Mr. A. Bakerspigel for preparing the unstained permanent mounts illustrated in FIGS. 1–7. The method is described in MYCOLOGIA **46**: 523–526. 1954.

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WOOD-STAINING FUNGI ASSOCIATED WITH BARK BEETLES IN ENGELMANN SPRUCE IN COLORADO

Ross W. Davidson [1, 2]

(with 3 figures)

Many bark beetles that attack conifers have wood-staining fungi (especially of the *Ophiostomataceae*) closely associated with them (**1**, **8**, **9**, and **13**), which may be important in the killing of trees after attack (**2**, **11**, and **12**). In 1936 Rumbold (**13**) described *Ceratostomella piceaperda* as a close associate of the bark beetle, *Dendroctonus piceaperda* Hopk., attacking *Picea glauca* (Moench.) Voss in eastern Canada. Since the imperfect stage of this fungus belongs in the genus *Leptographium* (**14**) it may now be considered a member of the genus *Grosmannia* as proposed by Goidanich (**5**). A somewhat similar fungus is closely associated with *D. engelmanni* Hopk. in the Rocky Mountain area. The present paper describes this fungus and several others of minor importance in beetle-attacked Engelmann spruce (*Picea engelmannii* Parry).

Leptographium associated with Dendroctonus engelmanni

First in 1945 and subsequently during the period 1950 to 1953 the writer often isolated a wood-staining fungus from *Picea engelmannii* recently attacked by *Dendroctonus engelmanni* Hopk. in western Colorado. The fungus is a *Leptographium* sp. (Fig. 1, A, B) and has also been isolated from pupating larvae and young adults of *D. engelmanni*. It seems to be closely and consistently associated with the insect. In the sapwood the fungus causes a light gray stain, which has been observed in all examined trees known to have been killed by the insect. The stain may be in streaks or small or large patches but only in areas of the trunk where the insects have invaded the bark, progressing from the entrance galleries. It penetrates the sapwood to a radial depth of 3 to

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5 inches. The fungus causes a dark gray stain in sapwood of lodgepole pine (*Pinus contorta* Loud.) attacked by the same bark beetle.[3] The *Leptographium* has been isolated from sapwood of trees, both spruce and pine, 5 to 6 years after they were killed by the beetles.

Perithecia have never been observed in cultures either on blocks of wood or other media, such as corn meal, malt, or potato dextrose agar. While perithecia of the *Ophiostomataceae* do occur in the main insect galleries, ascospore cultures from them show that they are not connected with *Leptographium*.

Comparison with other species of *Leptographium*.—Several species of *Leptographium* have been described, of which only those most closely related to the Engelmann spruce one need be considered here. The conidia of *L. lundbergi* Lag. & Mel. (**7**) are larger, conidiophores are shorter and more branched, and growth characteristics differ from those of the Engelmann spruce fungus. Of the three other European species, *L. repens* (*Grosmannia repens* Goid.) (**5**) has larger conidiophores and *G. penicillatum* (Gros.) Goid. and *G. polonicum* (Siem), both from *Ips typhographus* in *Picea excelsa* (**15**), have larger conidia and the conidiophores are not commonly in groups as are those of this species. The North American species *G. piceaperda* (Rumb.) Goid.? (**13**) has slightly larger conidia and conidiophores and perithecia consistently develop in the cultures. Some isolates of the *Leptographium* sp. from western white pine (**4**, **6**) are morphologically similar to the Engelmann spruce fungus. The conidia and conidiophores, as indicated by Hubert's (**6**) measurements of isolate No. 77, of the white pine fungus are slightly larger than is indicated in this paper. However, examination of a number of isolates from western white pine shows that there is considerable variation among them and it is beyond the scope of this paper to try to incorporate those isolates all or in part with the species described here. The Engelmann spruce *Leptographium* is, therefore, described here as a new species.

Leptographium engelmanii sp. nov. FIG. 1, A, B.[4]

Conidiophora abundantia, alia per superficiem ligni griseo-tincti dense effusa, alia in massis densis in cuniculis insectorum vel in stratis in parietibus camerorum puparum dipositis, saepe 2–4 caespitosa, brunnea, inte dum ramosa, 40–250 μ longa, 3–7 μ crassa; conidia in ramis penicillatis oriunda, in massa mucilaginos a in apicibus conidiophororum agglutinata, hyalina, unicellularia, oblonga vel ad apicem

[3] Lodgepole pine is not normally attacked by *D. engelmanni* but may be attacked and killed when it occurs in a mixture with heavily infested spruce.

[4] The Latin descriptions were prepared by Edith K. Cash, Mycologist, U. S. Department of Agriculture, Research Service.

unum aliquantus attenuata, 2–5 × 1.5–2 μ, conidia in hyphis directe oriunda maioria 4–5 × 2–3.5 μ.

Hab.: in ligno et cortice *Piceae engelmannii* e *Dendroctono engelmani* invasae, Colorado.

Conidiophores abundant, forming a dense gray growth over the surface of light to dark gray stained sapwood or in dense masses in insect tunnels or arranged in layers on walls of pupal chambers, often in groups

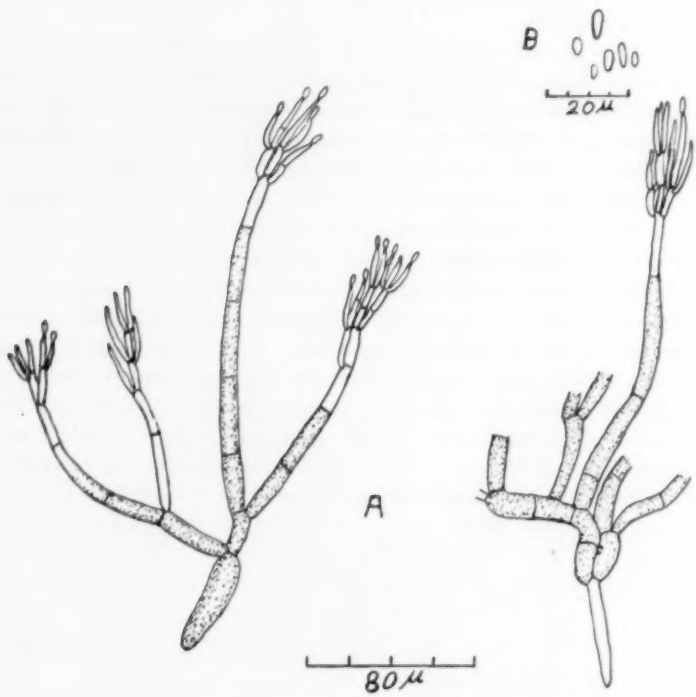

FIG. 1. *Leptographium engelmannii*. A. Groups of young conidiophores. B. Conidia.

of 2 to 4 from the same basal hypha, brown, sometimes branched, 40 to 250 μ long by 3 to 7 μ thick; conidia on penicillate branches and forming mucillagenous mass on tip of conidiophores, hyaline, one celled, oblong or somewhat smaller at one end, 2–5 × 1.5–2 μ, conidia formed directly on hyphae somewhat larger or about 4–5.5 × 2–3.5 μ.

Growth on 2 per cent malt agar medium about 40 mm diameter in 5 days at room temperature. "Saccardo's Olive" to "Mouse Gray,"

with raised, medium dense growth of conidiophores and mycelium partly obscuring the substratum.

On sapwood and bark of *Picea engelmannii* Parry dying from attack by *Dendroctonus engelmanni* Hopk. bark beetles, White River National Forest, Colorado, Grand Mesa National Forest, Colorado, and Routt National Forest near Steamboat Springs, Colorado, in 1945, 1947, 1950, 1951, 1952, and 1953 (Type F.P. *70793*). (Type deposited in the national fungus collections, Plant Industry Station, Beltsville, Maryland.) Also isolated from stained sapwood of *Pinus contorta* Doug. attacked by *D. engelmanni* in White River National Forest.

OTHER SPECIES OF STAINING FUNGI IN ENGELMANN SPRUCE

Perithecia belonging to the family *Ophiostomataceae* have repeatedly been observed in bark and sapwood of *Dendroctonus engelmanni*-infested spruce. Cultures from ascospores of these perithecia reveal that two main species are present. One of these is a form of *Endoconidiophora coerulescens* Münch. The characteristically hairy type perithecia of this species were present in small numbers in frass of the main entrance galleries of *D. engelmanni* and on ends of recently cut spruce logs. Cultures are similar in general to those described for *E. coerulescens* by Lagerberg et al. (**7**). It produces the amyl acetate odor which is characteristic of *E. coerulescens*.

A smooth type of perithecium is commonly present in the entrance galleries of *D. engelmanni* within the first year after the initial insect attack. The bases of the perithecia are often embedded in the bark at the edge of the galleries with the long necks protruding into the galleries. These are usually mixed with the abundant conidiophores of *Leptographium*. Cultures developed from ascospores revealed that this perithecial fungus has a *Graphium* type of imperfect stage. The presence of the perithecia in the entrance galleries suggests that it may also be closely associated with *D. engelmanni* along with *Leptographium* but such an association has not been proved by isolations from the beetles.

A third type of perithecial form has been obtained in culture from an adult *D. engelmanni* and once from an adult *Ips hunteri* ? beetle that was taken from an Engelmann spruce log also infested with *D. engelmanni*. This species has also been isolated from *Monochamus* collected in Canada. Additional information will be needed before it can be determined whether the fungus is definitely associated with bark beetles.

The latter two perithecial fungi are described as new species of the genus *Ophiostoma* and the *Endoconidiophora* will be considered as a form or variety of *E. coerulescens* and not given a distinct name.

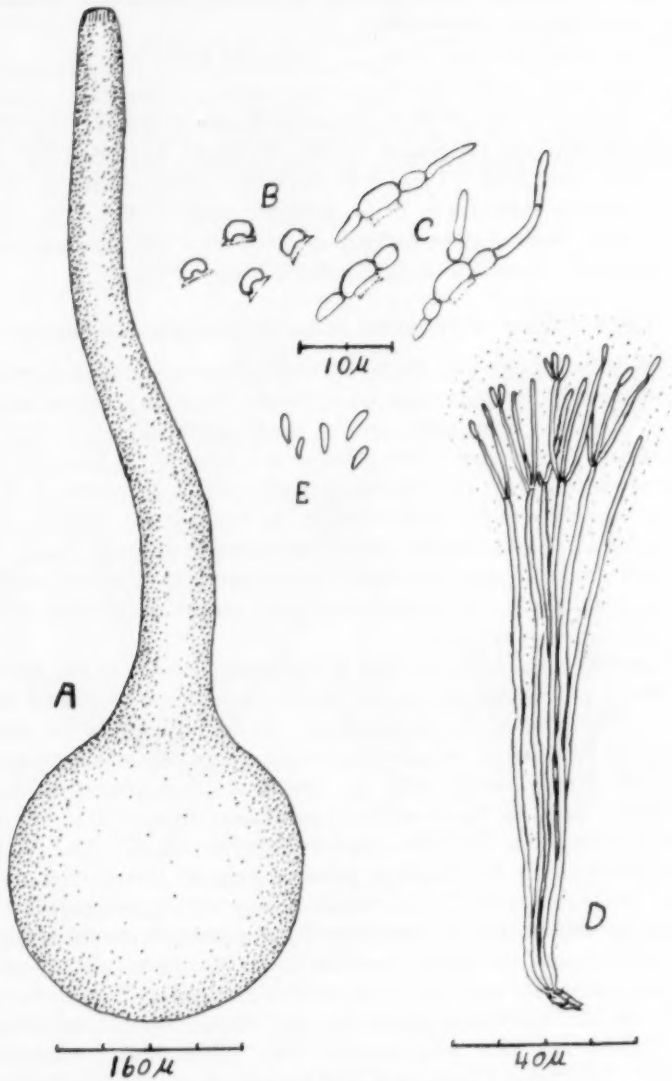

FIG. 2. *Ophiostoma truncicola*. A. Perithecium. B. Ascospores. C. Germinating ascospores. D. Small group of conidiophores, synnema. E. Conidia.

Ophiostoma truncicolor sp. nov. FIG. 2, A–E.

Perithecia atra, glabra, sphaerica vel subsphaerica, 225–300 μ in diam., ad superficiem porticularum insectorum disposita vel in cortice immersae, rostris protrudentibus, atris, distortis curvatis rectisve, 400–1200 μ longis, ad basim 50–75 μ, ad apicem 30–50 μ crassis, ciliis ostiolaribus carentibus; ascosporae in cirrhis tenuibus emergentes et in massam vixcidam ad apicem rostri confertae, hyalinae, unicellulares, 3–4.2 × 2.2–3.2 μ, in hyphis hyalinis vel in massis humidis albis ex apicibus synnematium oriunda; synnemata plerumque alba, interdum atropedicellata, 20–75 μ alta, 10–35 μ lata.

Hab.: in porticulis *Dendroctoni engelmanni* in ligno *Piceae engelmannii*, Colorado.

Perithecia black, smooth, base sperical or nearly so, 225–300 μ diameter, on surface of main galleries or in insect frass or embedded in bark with necks protruding into galleries; necks black, crooked, curved or straight, 400–1200 μ long, 50–75 μ thick at base and 30–50 μ thick at tip, with no bristles around ostiole; ascospores in fine tendrils in gelatinous matrix and collecting in sticky mass at tip of perithecial beak, hyaline, one celled, 3–4.2 × 2–2.6 μ, kidney or half-moon shaped, with inconspicuous gelatinous sheath, germinating in 20 to 40 hours on corn meal agar.

Conidia hyaline, one celled, broader at one end, 4–7 ×2.2–3.2 μ, on hyaline hyphae or in wet white masses on top of synnema (*Graphium*-like); synnema mostly white, sometimes with black stalks, variable in size but mostly small and short stalked, 20–75 μ high by 10–35 μ wide.

Cultures white at first, turning light gray to dark gray after 7 days; growth about 28 mm diameter on malt agar in 5 days at room temperature.

In galleries of *Dendroctonus engelmanni* Hopk., which were attacking and killing *Picea engelmannii* Parry. On Arapaho National Forest southeast of Kremmling, Colorado, collected March 1953 (Type F.P. *70792*) and from beetle-infested logs collected near Rabbit Ears Pass, Colorado, February 1954.

Ophiostoma bicolor Davidson & Wells, sp. nov. FIG. 3, A–D.

Perithecia subrosea usque pallide brunnea, sphaerica, 240–360 μ in diam., rostris atro-brunneis usque atris, 500–1200 μ vel longioribus, ad basim 50–70 μ, ad apicem 18–40 μ crassis, ciliis ostiolaribus carentibus; ascosporae ad apices rostrorum in filis irregularibus nec in guttulis sphaercis confertae, in massis flavidulae, rectangulares, 3.5–6.0 × 2.5–4 μ, hyalinae; conidia pauca in hyphis directe oriundis, ovoideis usque cylindricis, 5–12 × 3–5 μ, hyalina, crasse tunicata.

Hab.: e ligno *Piceae engelmannii* e *Ipse hunteri* et *Dendroctono engelmanni* invasae, Colorado; etiam e *Monochamo scutellato* et *Abiete balsamea*, in Canada isolatum.

Base of perithecium light pink to light brown, spherical, 240–360 μ diameter, necks dark brown to black, variable in length from 500–1200 μ, sometimes longer, 50–70 μ thick at base and 18–40 μ at tip, with no

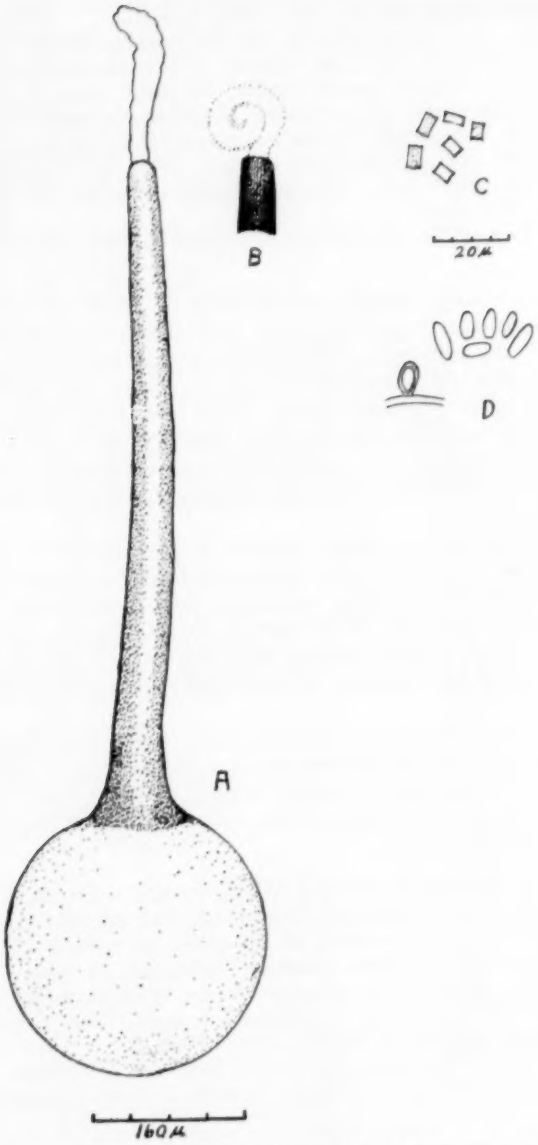

FIG. 3. *Ophiostoma bicolor*. A. Perithecium. B. Tip of perithecial neck. C. Ascospores. D. Conidia.

bristles around ostiole; ascospores collecting at tip of perithecial necks in irregular filaments or globules, yellowish in mass, box shaped, 3.5–6.0 ×2.5–4 μ, hyalin.

Only a few conidia seen, apparently formed directly on the hyphae, variable in size, ovoid to cylindrical, 5–12 × 3–5 μ, hyaline, thick walled.

Ascospore cultures grow about 50 mm in diameter in 4 days, white or hyaline at first, usually turning light to dark gray or brown after 8 days but sometimes with very little darkening; hyphae hyaline to light brown, up to 10 μ diameter and with right angled branches; perithecia start to develop in 2 weeks and mature in 3 to 4 weeks; their development may be stimulated by other fungi such as *Penicillium* sp.

Isolated from: *Ips hunteri* ? and *Dendroctonus engelmanni* Hopk. from *Dendroctonus* attacked *Picea engelmannii* Parry collected at Rabbit Ears Pass near Steamboat Springs, Colorado, July 1950 and January 1952 (Type F.P. *70711*). Also isolated from *Monochamus scutellatus* in 1951 and from *Abies balsamea* in 1952 and studied by Doreen Wells, Science Service, Ottawa, Ontario, Canada.

ENDOCONIDIOPHORA COERULESCENS Münch.

In beetle galleries in bark and on ends of logs recently cut from *Dendroctonus engelmanni* Hopk, attacked spruce (*Picea engelmannii* Parry) trees, Arapaho National Forest, southeast of Kremmling, Colorado, March 1953 and February 1954.

DISCUSSION

Ophiostoma truncicola is similar to *O.* (*Ceratocystis*) *galeiformis* (Bakshi) Mathiesen (**1**, **9**). There seem to be some differences in shape as ascospores, color of cultures, and size of conidia but general characteristics are similar. Bakshi states that his species has a *Leptographium*-like stage but his description and illustrations indicate that it is more typically a *Graphium* stage and does not belong to the *Grosmannia* group. It appears, rather, to belong with *O. ips*, *O. truncicola*, and *O. bicolor* to form a fairly distinct subgroup of the genus *Ophiostoma*.

Ophiostoma bicolor is distinct from any species known to the writer. It could possibly be an albino form of *O. ips* but color of mycelium, perithecia, and conidial stage differ so that at present it cannot be placed with this species.

The *Endoconidiophora coerulescens* is faster growing than the Douglas fir form (**3**), conidia are much less abundant, and differ in shape and size. Conidiophores are narrower and longer than those described by Lagerberg *et al* (**7**) and conidia are smaller. It is possible that *E. coerulescens* is an extremely variable species so that it will not be practical to describe and typify all varieties and forms.

SUMMARY

Four species of the *Ophiostomataceae* have been found associated with *Dendroctonus engelmanni* Hopkins in bark of dying *Picea engelmannii* Parry in Colorado. *Leptographium engelmannii*, the perfect stage of which has not been observed, is the most prominent and most consistently associated species, and is described as new. It causes a conspicuous stain in the sapwood of the insect-attacked trees. *Ophiostoma truncicola*, described as new, is frequently present in main galleries of the beetles. *Endoconidiophora coerulescens* Münch. is also sometimes present in the galleries and on sapwood of logs cut from beetle infested trees. The fourth fungus, *Ophiostoma bicolor* Davidson and Wells, described as new, has been isolated once from *D. engelmanni* adult beetle but was obtained from other sources such as *Ips hunteri* ? and *Monochamus scutellatus* (in Canada). All of these were obtained from bark beetle infested Engelmann spruce in Colorado except that the latter species (*O. bicolor*) was also obtained from Canada and from other hosts.

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NEW SPECIES AND VARIETIES OF ASPERGILLUS

DOROTHY I. FENNELL [1] AND KENNETH B. RAPER [2]

(WITH 8 FIGURES)

During the nine-year period since the publication of the "Manual of the Aspergilli" by Thom and Raper (**1945**), the writers have continued to make isolations of *Aspergillus* from soil and other natural sources. During this same period, collaborators in this country and abroad have submitted for identification many cultures isolated in their laboratories. Many interesting strains have been encountered, and a number of these seem to differ sufficiently from previously known species and varieties to warrant their description as new. The purpose of the present paper is to acquaint other mycologists with these hitherto undescribed fungi, and, in so doing, present a more complete picture of the abundant and cosmopolitan genus *Aspergillus* as it occurs in nature. In some measure, this communication may be considered as a supplement to the Manual.

Cultures isolated in the laboratory of the Culture Collection Unit, Northern Utilization Research Branch, were obtained from soil-streak plates on hay-infusion agar as described by Thom and Raper (**1944**). Subsequent to their isolation, all cultures were grown upon a wide variety of agar substrata and at different temperatures of incubation. The following agar media, previously recommended (Thom and Raper, **1945**; Raper and Thom, **1949**), were utilized in the comparative examination of new isolates and representative strains of recognized species: Czapek's solution, high-sugar Czapek's, steep-enriched Czapek's malt extract, potato dextrose, corn meal, and hay infusion. Additionally, strains which failed to produce abundant conidial structures were grown upon a very concentrated agar medium (40.0 per cent sucrose, 2.0 per cent malt extract, and 0.5 per cent yeast extract) recommended by Harrold (**1950**) for cultivating species of *Eremascus*.

In so far as possible, species and varietal descriptions are based upon cultural characteristics and details of morphology as these are exhibited

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on Czapek's solution and malt extract agars. Where enhanced development of conidial structures has been realized upon other substrata, the descriptions incorporate data obtained from these sources, also.

Color references are based upon plates in Ridgway's "Color Standards and Color Nomenclature" (**1912**).

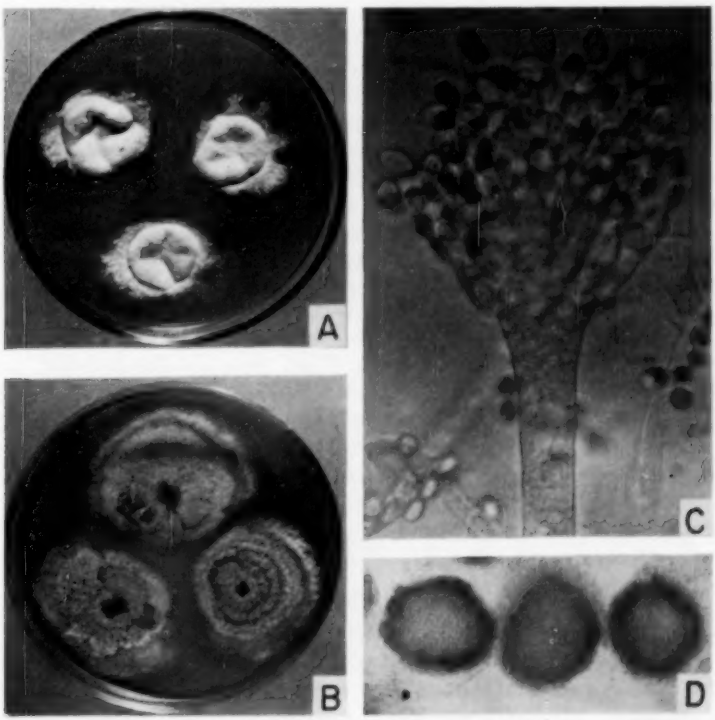

FIG. 1. *Aspergillus paradoxus;* NRRL 2162. *A* and *B*, Colonies on Czapek's solution and malt extract agars, respectively. *C*, Conidial head with single series of sterigmata and elliptical, delicately echinulate conidia, × 700. *D*, Sclerotium-like bodies with enveloping, interwoven sterile hyphae, × 55.

Aspergillus paradoxus sp. nov.

Coloniae in agaro Czapekii rapide crescentes, massas crassas myceliares floccosas pallide flavidulas efficientes et tarde conidiophora pallide cyaneo-viridia producentes, reverso et agaro flavo-aurantiis, odore gravi, mucido, exsudato flavo; capitula conidica laxe columnaria, 150–200 × 75–100 μ, e conidiophoris fragilibus plerumque 1–2 mm, interdum usque 1 cm longis, 12–20 μ in diam., tenui-tunicatis, subtiliter asperatis, septatis oriunda; areae vesiculosae vix inflatae, subclavatae, plerumque 15–25 μ in diam.; sterigmata uniseriata, pauca, plerumque 10–12 × 3.5-

5.5 μ; conidia ovoidea, subtiliter echinulata, 5.5–6.5 × 4.0–5.0 μ; massae compactae cellularum rotundarum, crasse tunicatarum plerumque 250–450 μ in diam., comparative firmae, subsclerotioideae, in partibus coloniae in numero variabiles productae.

In culturis e fimo ferae marsupialis "opossum" dictae, New Zealand.

Colonies on Czapek's solution agar (FIG. 1A) at room temperature (24° C) growing rapidly, 7–8 cm in 10 days to 2 weeks, with margin entire or somewhat dissected, quickly developing as a deep flocculent mass in light yellow shades near Marguerite Yellow to Primrose Yellow (Ridgway, Pl. XXX), bearing limited to fairly abundant conidial structures primarily from the submerged mycelium. Conidial heads developing tardily, small, very pale blue-green in color, seldom influencing the pigmentation of the colony; reverse and agar in bright yellow-orange shades near Lemon Chrome to Aniline Yellow in age (Ridgway, Pl. IV); odor very pronounced, penetrating, suggesting that of a root cellar; exudate limited to abundant, in clear yellow shades. Conidial heads loosely columnar, up to 150–200 × 75–100 μ, commonly smaller, borne on long, frail conidiophores, which collapse quickly upon exposure to air and in mounting fluids; conidiophores variable in length, commonly from 1–2 mm but sometimes up to 1 cm, strongly phototrophic, thin-walled, delicately roughened, several times septate, commonly 12–20 μ in diameter, terminating in vesicular areas showing only slight enlargement (FIG. 1C); vesicles 15–25 μ, seldom larger; sterigmata in a single series, limited in number, variable in size, commonly 10–12 × 3.5–5.5 μ, borne only on the terminal portion of the vesicular areas, with tips often incurved and producing conidia in a loose column; conidia ovoid, delicately echinulate, 5.5–6.5 × 4.0–5.0 μ (FIG. 1C).

Compact masses of rounded thick-walled cells (FIG. 1D) enveloped by interwoven sterile hyphae are characteristically produced in greater or lesser abundance, oftentimes concentrated in limited sectors and produced more abundantly at elevated temperatures, commonly 250–450 μ in diameter, comparatively firm, constituent cells, variable in size, parenchyma-like, representing neither hülle cells nor the heavy-walled sclerenchyma-like elements of true sclerotia as seen in the *Aspergillus niger* and other groups.

Colonies on malt extract agar (FIG. 1B) growing rapidly as on Czapek's but tending to produce less flocculent mycelium; "sclerotia" produced in somewhat greater abundance. Pigmentation of colony and reverse in yellow shades but less brilliant than on Czapek's agar.

The binomial *A. paradoxus* is chosen because of the conflicting structural characteristics which are not consistent with those of any one currently recognized group of Aspergilli.

Type strain, NRRL *2162*, was received in January, 1948, from Dr.

J. H. Warcup, University of Cambridge, England, as his No. *A-28* isolated from oppossum dung, Wellington, New Zealand.

Aspergillus paradoxus represents an enigma. Its true relationships remain in doubt. Although the conidial heads most nearly approximate those of *A. clavatus* Desm., they differ strikingly from typical isolates of this species. However, occasional strains of the latter species have been encountered wherein the conidial heads rather closely approximate those of *A. paradoxus* in pattern and dimensions. Strain NRRL *1*, originally from Professor Westerdijk, represents such an example and is discussed and illustrated in the "Manual of the Aspergilli" (Thom and Raper, **1945**, pp. 94–95, Fig. 22).

The conidiophores of *A. paradoxus* are longer than those of any known strain of *A. clavatus*. They are, however, exceeded in length by *A. giganteus*, the other recognized member of the *A. clavatus* group. In spite of the similarities noted above, we find it difficult to assign *A. paradoxus* to the *A. clavatus* group since it produces conidiophores which are delicately roughened and, more particularly, since it produces abundant and conspicuous masses of rounded, thick-walled cells that superficially resemble sclerotia. The species is represented by the type strain only; it is hoped that additional cultures will be encountered and that examination of these will provide additional criteria which will clarify the true relationships of this disconcerting species.

Aspergillus aureolus sp. nov.

Coloniae rapide in agaro Czapekii crescentes, leniter radiatim furcatae et subzonatae, flavae, capitula conidica pauca pallide cyaneoviridia et perithecia abundantia gerentes, reverso flavo, exsudato abundanti et hyalino, odore non gravi; capitula conidica columnaria, longitudine variabilia, circa 25–30 μ in diam.; conidiophora sinuosa, brevia, generaliter minus quam 50 μ longa, 2.5–4.5 μ in diam., glabra, hyalina usque viridula; vesiculae lageniformes, 6.0–9.0 μ in diam.; sterigmata uniseriata, conferta, 7.0–8.0 $\times$ 2.5–2.7 μ; conidia globosa vel subglobosa, 3.0–3.3 μ in diam., subtiliter echinulata; perithecia globosa vel subglobosa, plerumque 250–350 μ in diam., pallide citrinula, fragilia, peridio hyphis intertextis sicut in *A. fischeri* composito; asci octospori, globosi usque ovoidei, 10–12 μ in diam.; ascosporae hyalinae, lenticulares, cristis duobus prominentibus ornatae, superficiebus convexis conspicue echinulatis, 6.0–7.0 $\times$ 4.4–5.0 μ.

In culturis e solo, Liberia.

Colonies on Czapek's solution agar (FIG. 2A) spreading rapidly, 7.0–8.0 cm in 10 days to 2 weeks at room temperature (24° C), consisting of a fairly tough basal felt overgrown by a loose flocculent mycelium, somewhat radially furrowed at colony center, slightly zonate, margins white becoming Maize Yellow through Apricot to Light Cadmium Yellow (Ridgway, Pl. IV) at colony center, producing very few conidial heads, light blue-green in color and not affecting the colony

appearance, perithecia produced throughout the colony but most abundantly in submarginal areas; reverse in conspicuous yellow shades, ranging from lightly colored to Yellow Ocher and Ochraceous Orange near Dresden Brown (Ridgway, Pl. XV), becoming brown in age and under

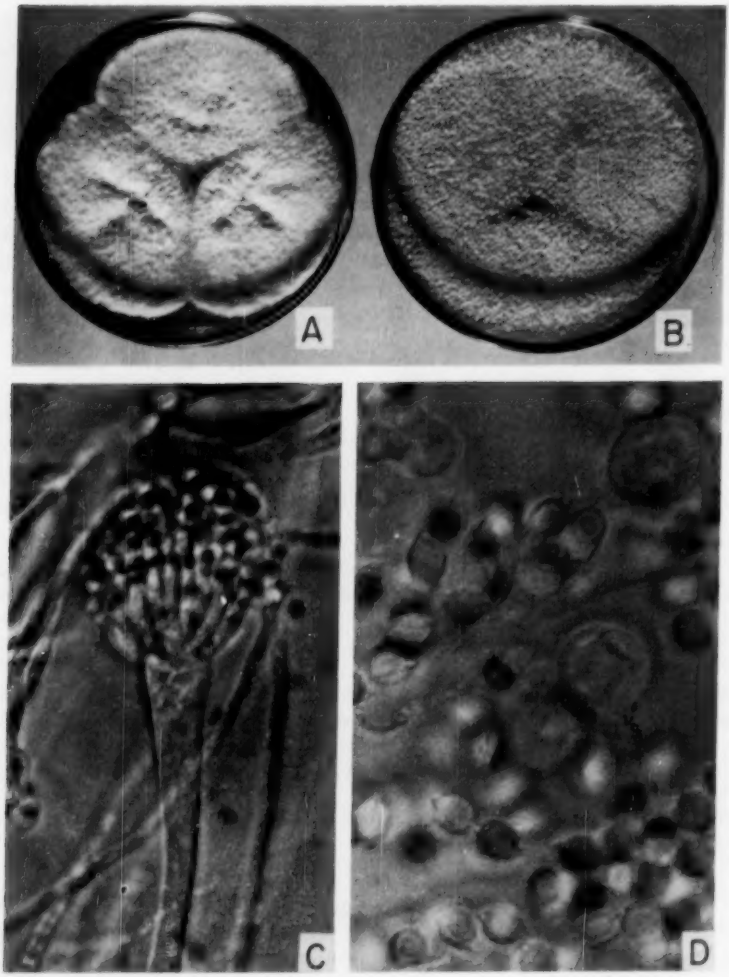

FIG. 2. *Aspergillus aureolus;* NRRL 2244. *A* and *B*, Colonies on Czapek's solution and malt extract agars, respectively. *C*, Conidial head showing crowded sterigmata in a single series, × 1070. *D*, Asci and ascospores, the latter with two prominent equatorial crests and convex surfaces conspicuously echinulate, × 1160.

areas of heaviest perithecial development; exudate clear, fairly abundant, embedded in the mycelium in rather large droplets; odor not pronounced. Conidial heads (FIG. 2C) borne on short conidiophores from aerial hyphae, loosely columnar, about 25–30 μ in diameter; conidiophores somewhat sinuous, short, commonly less than 50 μ in length, 2.5–4.5 μ in diameter, smooth-walled, uncolored to slightly greenish in terminal areas; vesicles flask-shaped, mostly 6.0–9.0 μ in diameter; sterigmata in a single series, rather crowded, variable in size, mostly 7.0–8.0 × 2.5–2.7 μ; conidia globose to subglobose, mostly 3.0–3.3 μ in diameter, delicately echinulate. Perithecia globose or nearly so, variable in size, ranging from 175–500 μ, mostly 250–350 μ, pale lemon yellow and surrounded by loose wefts of dark golden-yellow hyphae, easily crushed, peridium thin and consisting of interwoven hyphae as in *A. fischeri;* asci (FIG. 2D) 8-spored, globose to ovoid, 10–12 μ diam.; ascospores (FIG. 2D) hyaline, lenticular, with two prominent equatorial crests and with convex surfaces conspicuously echinulate, 6.0–7.0 × 4.4–5.0 μ.

Colonies on malt extract agar (FIG. 2B) spreading broadly, 8–10 cm in 10 days to 2 weeks, consisting of a loose flocculent mycelium bearing abundant perithecia throughout, in yellow-orange shades from Warm Buff to almost Deep Chrome (Ridgway, Pl. III) at colony centers; conidial structures lacking or very limited in number and not affecting colony appearance; perithecia very abundant and with enveloping mycelium imparting to the colony its characteristic pigmentation and texture; reverse in orange-red shades near Orange Rufous to Sanford's Brown (Ridgway, Pl. II).

Colonies on Harrold's malt extract-sucrose-yeast extract agar (**1950**) growing well, 2.0–3.0 cm in one week, producing a very dense stand of conidial structures which gives the colony a light bluish-green color near Glaucous Green (Ridgway, Pl. XXXIII), perithecia not developing within two weeks.

The binomial *A. aureolus* is chosen because of the brilliant golden color of the growing colonies on common laboratory substrata.

Type strains, NRRL *2244* and NRRL *2391,* were isolated during the spring of 1950 from samples of soil submitted by C. F. Charter, West African Cacao Research Institute, Tafo, Gold Coast, Africa, and by Prof. J. T. Baldwin, Monrovia, Liberia, respectively.

Aspergillus aureolus obviously belongs in the series with *A. fischeri* Wehmer (**1907**). The pigmentation and pattern of the conidial structures closely approximate those of the latter species, albeit they are produced much more sparingly on most substrata. Perithecia, likewise, closely resemble those of *A. fischeri* in form and texture. They differ markedly from *A. fischeri,* however, in the bright pigmentation of the

loose aerial hyphae which characteristically envelop the perithecia in all members of this series. The ascospores, while showing the general pattern of *A. fischeri,* differ from these in having convex surfaces echinulate rather than ridged.

In their treatment of the *A. fischeri* series, Thom and Raper (**1945**) recognized only Wehmer's species but called attention to the presence of considerable variation among the isolates which they had observed. *A. malignus* Lindt was regarded as probably synonymous with *A. fischeri.* Since the publication of the Manual, George Smith, London School of Hygiene and Tropical Medicine, has studied and distributed a culture which he apparently regards as representing Lindt's fungus. This differs from *A. fischeri* in its classic and typical form by producing fewer conidial structures, a relatively heavier crop of perithecia, a reduced amount of cottony aerial mycelium, and ascospores with convex walls showing prominent spine-like projections and wide equatorial crests which characteristically appear angular when spores are observed in face view. Very recently, Yuill (**1953**) has discussed and illustrated a culture (IMI *16061*) which apparently duplicates the strain earlier received from Smith. The character of the ascospores of *A. aureolus* might be interpreted to indicate a relationship somewhat intermediate between *A. fischeri* as described and illustrated by Thom and Raper (**1945**, Fig. 38) and *A. malignus* Lindt as currently recognized by both Smith and Yuill. Yuill has recently described a new species in the *A. fischeri* group, *A. quadricinctus* (**1953**), which shows four equatorial bands comparable to *A. quadrilineatus* in the *A. nidulans* group. Thus it appears that the *A. fischeri* series, like the *A. glaucus* and *A. nidulans* groups, represents not a single species but a complex of ascosporic forms wherein species differ one from the other in habits of growth, ascospore patterns, etc.

ASPERGILLUS FISCHERI var. **glaber** var. nov.

A typo differt peritheciis minoribus et superficiebus convexis ascosporarum levibus.

Colonies on Czapek's solution agar essentially duplicating those of the species, with texture and pigmentation dependent upon the relative abundance of perithecia and conidial heads. This relationship varies with the strain and the temperature of incubation. Conidial heads typical of the species and produced more abundantly at elevated temperatures. Perithecia abundantly produced throughout the colony or concentrated in localized areas or sectors, globose or nearly so, mostly 150–200 μ in diameter; asci 8-spored, typical of the species; ascospores lenticular, uncolored, with two prominent equatorial crests and with

convex surfaces smooth, mostly 6.5–7.5 × 4.5–5.0 μ, with crests about 1.0–1.5 μ wide.

Type strain, NRRL *2163*, was received in September, 1947, from Prof. G. W. Martin, University of Iowa, as his No. *6354*, isolated from rubber scrap from old tires. The species is represented also by NRRL *2392*, received in September, 1952, from Dr. J. H. Warcup, Waite Agricultural Research Institute, Adelaide, South Australia, as his strain No. SA *14*, isolated from garden soil. This latter strain differs from the type in producing less-rapidly growing colonies and ascospores that are somewhat smaller, 5.5–6.6 × 4.4–5.0 μ, with narrower, more widely separated crests.

Aspergillus violaceus sp. nov.

Coloniae nonnihil restricte in agaro Czapekii crescentes, mycelio dense coacto compositae et perithecia abundantia producentes, lilacinae usque violaceo-griseae, reverso brunneo, odore parvo; fructificationes conidicae non visae; perithecia globosa vel subglobosa, 100–200 μ in diam., a cellulis "hülle" dictis globosis laxe involuta; asci octospori, ovoidei usque globosi, circa 12–14 × 10–12 μ; ascosporae valde violaceae, lenticulares, cristis aequatorialibus duobus humilibus ornatae, superficiebus convexis echinulatis, 5.5–6.5 × 4.0–5.0 μ; fructificationes conidicae in agaro foeni sparsae, parvae vel incompletae; conidiophorae hyphis aeriis oriunda, glabra, perbrevia, 30–50 × 3–4 μ, subsinuosa, hyalina vel subhyalina, areis vecicularibus plerumque 5–6 μ diam.; sterigmata primaria pauca, plurimum 6.0–7.5 × 3.0–3.5 μ, secondaria circa 5.0–6.0 × 2.0–2.5 μ; conidia globosa vel subglobosa, pallide viridia, glabra vel delicater asperata, plerumque 2.8–3.3 μ in diam.

In culturis e solo, Africa occidentalis.

Colonies on Czapek's solution agar (FIG. 3A) at room temperature (24° C) growing somewhat restrictedly, with thin margin, white, attaining a diameter of 3.0–3.5 cm in 10 days to 2 weeks, consisting of a closely felted mycelium, irregularly buckled and wrinkled, commonly splitting in colony center, producing abundant perithecia upon and within a mycelial matrix, ranging in color from lilac to violet-gray shades (Ridgway, Pl. LII); reverse in brownish drab shades (Ridgway, Pl. XLV); odor negligible. Conidial structures not observed. Perithecia crowded, forming an almost continuous layer at intercolony margins and within the older colony areas, globose or nearly so (FIG. 3D), variable in size, ranging from 100–200 μ, surrounded by a loose envelope of hülle cells and interwoven hyphae; asci (FIG. 3F) 8-spored, ovoid to globose, about 12–14 × 10–12 μ; ascospores (FIG. 3F) in deep violet-blue shades, lenticular, with two very low equatorial crests and convex surfaces echinulate, with roughenings commonly arranged in a regular pattern, 5.5–6.5 × 4.0–5.0 μ.

Colonies on malt extract agar (FIG. 3B) growing more rapidly, 6.5–7.5 cm in 10 days to 2 weeks, plane, consisting of a dense layer of

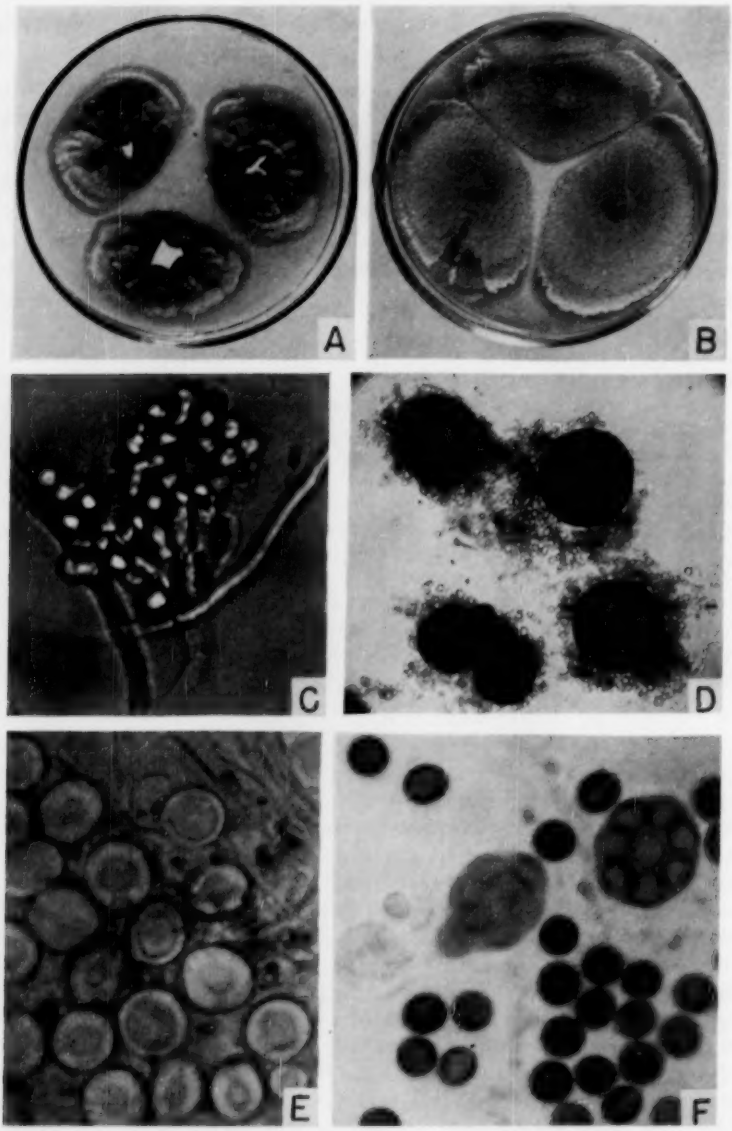

FIG. 3. *Aspergillus violaceus;* NRRL 2240. *A* and *B*, Colonies on Czapek's solution and malt extract agars, respectively. *C*, Conidial head showing double series of sterigmata, × 1070. *D*, Mature perithecia, uncrushed, with enveloping layers of globose hülle cells, × 55. *E*, Globose hülle cells, characteristic of this species and other members of the *A. nidulans* group, × 580. *F*, Immature asci and ascospores with echinulate convex walls and low equatorial crests, × 1160.

perithecia with a minimum of aerial mycelium, in olive-buff shades (Ridgway, Pl. XL); odor negligible; reverse in dull light-brown shades. Conidial structures lacking. Perithecia somewhat larger than on Czapek's agar, occasionally up to 300 μ in diameter; asci and ascospores as described above.

Colonies on hay-infusion agar thin, spreading, attaining a diameter of 6.5–7.0 cm in 10 days to 2 weeks, with vegetative mycelium largely submerged and with perithecia abundant but not forming a continuous layer. Conidial structures (FIG. 3C) scattered, small and commonly fractional, not affecting the colony appearance. Conidiophores arising primarily from aerial hyphae, smooth-walled, very short, 30–50 μ in length by 3.0–4.0 μ in diameter, somewhat sinuous, thin-walled, hyaline or nearly so, terminating in rounded and somewhat enlarged vesicular areas mostly 5.0–6.0 μ, primary sterigmata few in number, borne on the upper part of the vesicle only, variable in dimensions, mostly 6.0–7.5 $\times$ 3.0–3.5 μ; secondary sterigmata about 5.0–6.0 $\times$ 2.0–2.5 μ, bottle-form, bearing conidia in short chains; conidia globose or nearly so, light green in color, smooth, or delicately roughened, mostly 2.8–3.3 μ in diameter.

The binomial *A. violaceus* is chosen because of the violet-blue color of the ascospores.

Type strain, NRRL *2240,* isolated in May, 1950, from a sample of soil sent to us by C. F. Charter, West African Cacao Research Institute, Tafo, Gold Coast, Africa.

Aspergillus violaceus is based upon a single culture which we believe differs sufficiently from any previously observed strain of any known species of *Aspergillus* to warrant species recognition. We have not succeeded in obtaining conidial structures upon the substrata commonly employed for the study and identification of *Aspergilli* (e.g. Czapek's and malt extract agars). Nevertheless, the morphology of conidial structures, as determined from the limited and generally minute heads which we observed on hay-infusion agar, indicates that this culture represents a new member of the *A. nidulans* group. Such relationship is further corroborated by the appearance of the growing colonies, by the nature of the perithecia with their envelopes of globose hülle cells, and by the character and pattern of the ascospores.

The ascospores of *A. violaceus* clearly align the species with the *A. nidulans* group. Their violet-blue color and their ornamentations, which include two very low equatorial crests and conspicuous echinulation of their convex surfaces, serve, however, to distinguish them from those of currently recognized species. Whereas the pigment of the ascospores

in established species of the *A. nidulans* group changes from purple-red to orange-red with a change from an alkaline to an acid reaction, the ascospore pigment of *A. violaceus* changes from near indigo blue to purple under comparable conditions. Under no conditions have we observed a red pigmentation in the ascospores of *A. violaceus*.

ASPERGILLUS UNGUIS Emile-Weil & Gaudin, Thom & Raper **emend.**

The species description presented by Thom and Raper (**1939, 1945**) for *A. unguis* as a non-ascosporic member of the *A. nidulans* group is emended to include a strain which tardily produces perithecia in limited numbers. The sterile spicular hyphae which constitute a particularly

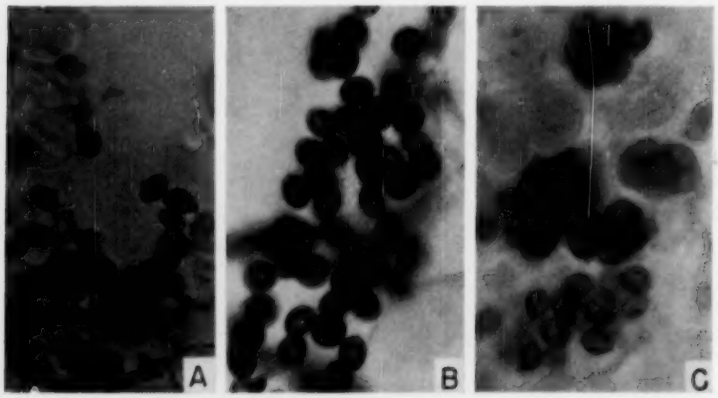

FIG. 4. *A*, Ascospores of *Aspergillus unguis* emend., NRRL 2393, showing two low equatorial crests and smooth convex walls, × 990. *B*, Ascospores of *A. nidulans* var. *acristatus*, NRRL 2394, showing smooth convex walls and entire absence of crests, × 990. *C*, Ascospores of *A. nidulans* var. *echinulatus*, NRRL 2395, with echinulate convex walls and prominent pleated equatorial crests, × 990.

distinctive characteristic of *A. unguis* are produced abundantly by the ascosporic strain. Structural elements of the conidial heads average slightly larger in this strain but do not differ substantially from *A. unguis* as previously known.

Perithecia are sparingly produced and have been observed only on malt extract agar, occurring generally near the margins of contiguous colonies, globose to subglobose, up to 200–250 μ in diameter, surrounded by a thin envelope of globose hülle cells; asci 8-spored, ovoid to subglobose, about 9.5–10.5 μ in diameter; ascospores (FIG. 4A) lenticular, purplish red, with two low equatorial crests and convex walls smooth, about 4.5–5.5 × 3.2–3.5 μ.

The ascosporic stage has been observed only in strain NRRL *2393*, received in December, 1948, from Dr. Elwyn T. Reese, Philadelphia Quartermaster Depot, as No. QM *25b*.

The discovery of occasional perithecia and ascospores in a culture with the structural pattern and general morphology of *A. unguis* raises some doubt concerning the validity of this species, particularly when the ascospores exhibit the general pattern of those present in *A. nidulans*. We believe, however, that the species *A. unguis* should be retained, at least for the present, to include the numerous strains belonging to the *A. nidulans* group which grow restrictedly on many substrata, produce long, sterile spicular hyphae and are commonly isolated from soil and from situations indicating at least secondary pathogenicity. Strain NRRL *2393* should be regarded, perhaps, as transitional between *A. unguis* and *A. nidulans*.

ASPERGILLUS NIDULANS var. **acristatus** var. nov.

A typo differt cristis aequatorialibus ascosporarum deficientibus.

Colonies on Czapek's solution agar growing well at room temperature (24° C), attaining a diameter of 5.5–7.0 cm in 10 days to 2 weeks, at first white, but becoming Olive Buff with the development of abundant perithecia with envelopes of hülle cells, approaching light grayish olive in age (Ridgway, Pl. XLVI), conidial structures generally duplicating those of the species, very limited in number, not affecting the colony appearance; conidiophores commonly showing conspicuous brownish encrustments. Perithecia globose, variable in size from 50–200 μ in diameter, but generally duplicating those of the species. Ascospores (FIG. 4B) orange-red in color, lenticular, showing neither equatorial crests nor surface markings of any kind but of the usual bivalve construction, mostly 4.5–5.0 × 3.5–4.0 μ.

Type strain, NRRL *2394*, received in November, 1952, from Colonel M. E. Sorte, Wright-Patterson Air Force Base, Dayton, Ohio, as his isolate No. *62X* from fabric exposed in New Mexico.

The variety differs from the species significantly only in the production of crest-free ascospores, hence the varietal name.

ASPERGILLUS NIDULANS var. **echinulatus** var. nov.

A typo differt colore roseo-cinnamomeo a peritheciis numerosis producto, capitibus conidicis atque peritheciis majoribus, et superficiebus convexis ascosporarum manifeste echinulatis.

Colonies on Czapek's solution agar growing well at room temperature (24° C), attaining a diameter of 4–5 cm in 10 days to 2 weeks, generally duplicating the species in appearance, but differing from it in develop-

ing a pronounced pinkish-cinnamon color due to the production of abundant perithecia and only limited conidial structures. Heads slightly larger than in typical representatives of the species; perithecia substantially larger, commonly 400 μ in diameter and occasionally up to 450 to 500 μ; asci 8-spored; ascospores (FIG. 4C) lenticular, red-orange in color, with two prominent pleated equatorial crests about 1.0 μ wide and with convex surfaces conspicuously echinulate rather than smooth.

Conidial structures produced abundantly on malt agar, evenly distributed throughout the colony.

Type strain, NRRL *2395*, received in October, 1950, from Juana Winitsky, Instituto de Microbiologia Agricola, Buenos Aires, Argentina, as culture No. *M–1–354*. Represented also by a strain isolated in April, 1944, from a sample of soil received from G. and C. Muskus, Caracas, Venezuela.

ASPERGILLUS VARIECOLOR var. **astellatus** var. nov.

A typo differt ascis lobatis, ascosporis maioribus, et cristis aequatorialibus ascosporarum angustioribus integris.

This variety differs primarily from the species *A. variecolor* as discussed and illustrated by Thom and Raper in their treatment of the *A. nidulans* group (**1939, 1945**) by producing ascospores with very wide but entire rather than stellate equatorial bands (FIG. 5B). As in the species, perithecia are typically borne on pseudostalks consisting of mounds of globose hülle cells and interwoven mycelium. Growth on Czapek's solution agar is very sparse with vegetative mycelium largely

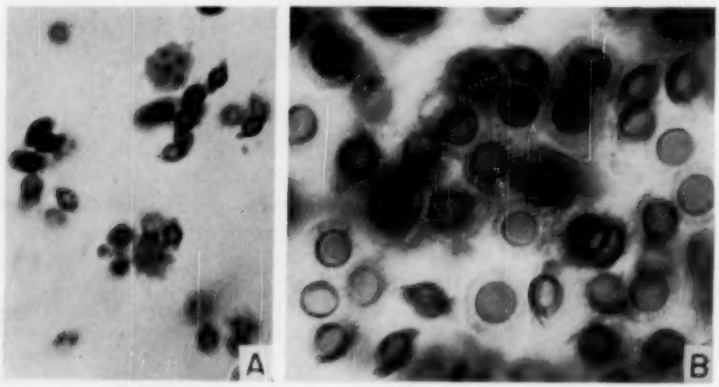

FIG. 5. *Aspergillus variecolor* var. *astellatus;* NRRL 2396. *A,* Immature asci showing lobed character apparently unique to this species, × 550. *B, Ascospores* showing wide, non-stellate crests, × 1050.

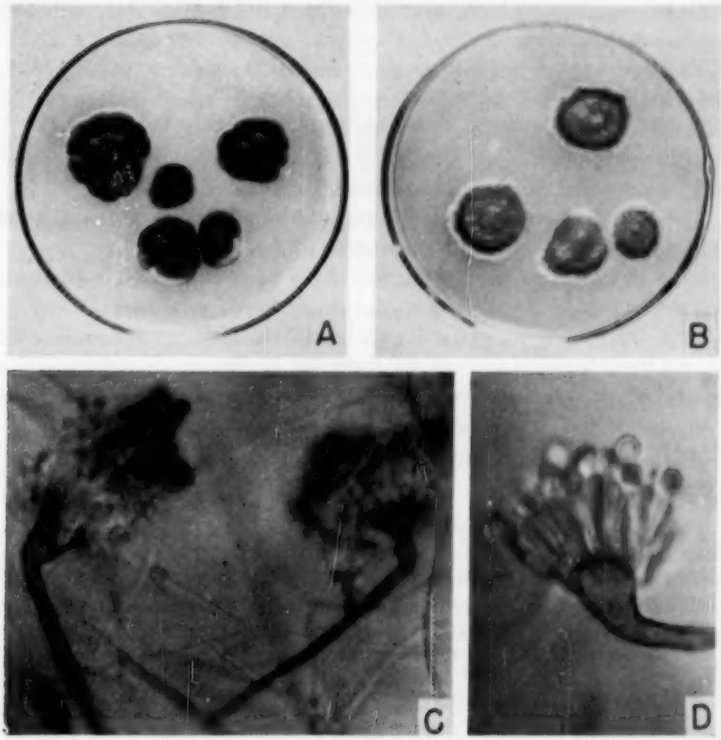

FIG. 6. *Aspergillus deflectus;* NRRL 2206. *A* and *B*, Colonies on Czapek's solution and malt extract agars, respectively. *C*, Conidial heads, showing characteristic angle at which vesicles are borne in relation to the conidiophores, and the pigmentation of stalks and vesicles, × 650. *D*, Conidial head showing greater detail, × 1240.

submerged as in the type strain of *A. variecolor*, NRRL *212*, (Thom and Raper, **1945**). Colonies on malt extract agar, and on Czapek's solution agar enriched with 1 percent steep liquor, grow more luxuriantly and produce perithecia in much greater abundance.

Conidial structures and perithecia of the new variety duplicate those of the species. The asci of this variety measure 10–13 × 14–18 μ and, unlike any other recognized ascosporic species of *Aspergillus*, appear conspicuously lobed during maturation (FIG. 5A). The ascospores of *A. variecolor* var. *astellatus* differ from the species not only in ornamentation but in dimensions. In *A. variecolor*, spore bodies measure 3.6–4.0 × 2.8–3.0 μ with crests up to 3.5 μ in width, whereas the spore

bodies of the variety measure 5.5–6.0 μ with completely undissected crests up to 2.8 μ wide.

The nutrient deficiency responsible for the sparse growth of *A. variecolor* var. *astellatus* on Czapek's solution agar can be partially corrected by replacing sucrose with glucose as a carbon source. The substitution of ammonium nitrate for sodium nitrate as a nitrogen source leads to increased production of conidial structure.

The proposed name is based upon the broad, undissected equatorial crests which characterize the ascospores of the new variety.

Two strains typifying the variety have been examined, both having been sent to us by Prof. G. W. Martin, University of Iowa. The first of these, NRRL *2396*, was received in March, 1946, as strain No. *6273* isolated from leaves (*Ilex* sp. ?) collected on Baltra (South Seymour) Island, Galapagos; the second, NRRL *2397*, was received in June, 1948, as strain No. *6368*, isolated on South Seymour Island, Galapagos, from a dead leaf.

Aspergillus deflectus sp. nov.

Coloniae restricte in agaro Czapekii crescentes, subzonatae et aliquantulum radiatim furcatae, mycelio lento basali fructificationes conidicas abundantes producenti compositae, areis centralibus murinis, exsudato abundanti, pellucido, reverso et agaro lateritiis, odore mucido; capitula conidica laxe columnaria, brevia, plerumque 25–30 μ in diam.; conidiophora brevia, subsinuosa, vulgo 40–50 × 2.5–3.5 μ, glabra, rubro-brunnea; vesiculae concolores, rotundatae vel lageniformes, typice axibus principalibus ad rectangulam oriundis, 5.5–6.5 μ in diam.; sterigmata biseriata, primaria 4.5–5.5 × 2.8–3.3 μ, secondaria 4.5–5.5 × 1.8–2.2 μ; conidia globosa vel subglobosa, irregulariter asperata, 3.0–3.4 μ in diam.

In culturis e solo, Brasilia.

Colonies on Czapek's solution agar (FIG. 6A) growing restrictedly, attaining a diameter of 1.5–2.0 cm in 10 days to 2 weeks at room temperature (24° C), very compact, consisting of a close-felted, tough, basal mycelium, slightly zonate; margins abrupt, somewhat radially furrowed; conidial structures abundantly produced, central colony area Mouse to Deep Mouse Gray (Ridgway, Pl. LI), growing margin near Congo Pink (Ridgway, Pl. XXVIII) from admixture of vegetative mycelium; exudate fairly abundant, clear, embedded in the mycelial mass as small droplets, not conspicuous macroscopically; reverse and agar in dull orange-red shades near Terra Cotta (Ridgway, Pl. XXVIII) becoming brown in age; odor fairly pronounced, moldy. Conidial heads evenly distributed, broadly columnar, mostly 25–30 μ in diameter, borne on short conidiophores from the aerial felt; conidiophores somewhat sinuous, mostly 40–50 μ in length, 2.5–3.5 μ in diameter, smooth-walled, reddish brown with pigmentation extending into the vesicles and sterigmata (FIG. 6C, D); vesicles rounded, flask-shaped, characteristically

borne at or near right angles to the main axes of the conidiophores (FIG. 6C, D), 5.5–6.5 μ in diameter, bearing sterigmata on the uppermost surfaces only; sterigmata in two series, primaries 4.5–5.5 × 2.8–3.3 μ, secondaries 4.5–5.5 × 1.8–2.2 μ, often abortive and failing to produce conidia; conidia globose to subglobose, 3.0–3.5 μ, with variable ornamentation, ranging from almost smooth to irregularly roughened; hülle cells not observed.

Colonies on malt extract agar (FIG. 6B) restricted as on Czapek, less heavily sporing and with conidial structures obscured by abundant loose sterile yellow mycelium, Old Gold to Buffy Citrine in color (Ridgway, Pl. XVI). Structural elements of conidial heads generally somewhat larger than on Czapek's with vesicles up to 8.5 μ in diam., secondary sterigmata up to 7.5 μ in length; conidia consistently produced, irregularly and coarsely roughened.

The binomial *A. deflectus* is chosen because of the conspicuous angle at which the conidial heads are borne on the conidiophores.

Type culture, NRRL *2206,* was isolated in April, 1949, from a sample of soil sent to us by Dr. A. Cury, Rio de Janeiro, Brazil.

This species is regarded as belonging in the group typified by *A. ustus.* In common with that species and closely allied forms, *A. deflectus* is characterized by conidial structures in dull-brown to gray shades and conidiophores which exhibit a conspicuous brown coloration. Additionally, pigmentation of the vegetative mycelium of this species, on Czapek's and other media, bears a striking resemblance to that of *A. ustus* var. *laevis* (Bloch.) Thom & Raper. Thus far we have not succeeded in demonstrating the presence of any type of hülle cell on any substratum employed. The absence of such large thick-walled cellular elements, which are characteristic of the *A. ustus* group, is outweighed by other characters which align it unmistakably with this group.

More than any other single character, the pattern of the conidial structure, which strikingly resembles a briar pipe (FIG. 6C, D), serves to distinguish the species.

Aspergillus silvaticus sp. nov.

Coloniae in agaro Czapekii bene crescentes, in areis centralibus elevatae, radiatim furcatae, stratum fere continuum cellularum "hülle" dictarum flavidularum et fructificationes conidicas virides producentes, reverso bubalino usque roseo, exsudatio hyalino, odore terreo; capitula conidica radiatim disposita, plerumque 200–300 μ in diam., e conidiophoris per stratum cellularum "hülle" protrudentibus, 250–270 μ longis, 5.5–8.5 μ in diam., glabris, brunnescentibus oriunda; vesiculae fere globosae brunnescentes, plerumque 14–17 μ in diam.; sterigmata biseriata, conferta, primaria 5.5–7.0 × 2.8–3.3 μ, secondaria 5.5–7.0 × 2.2–2.8 μ; conidia globosa vel subglobosa, valde flavo-viridia, conspicue asperata, maxima ex parte 3.0–3.5 μ in

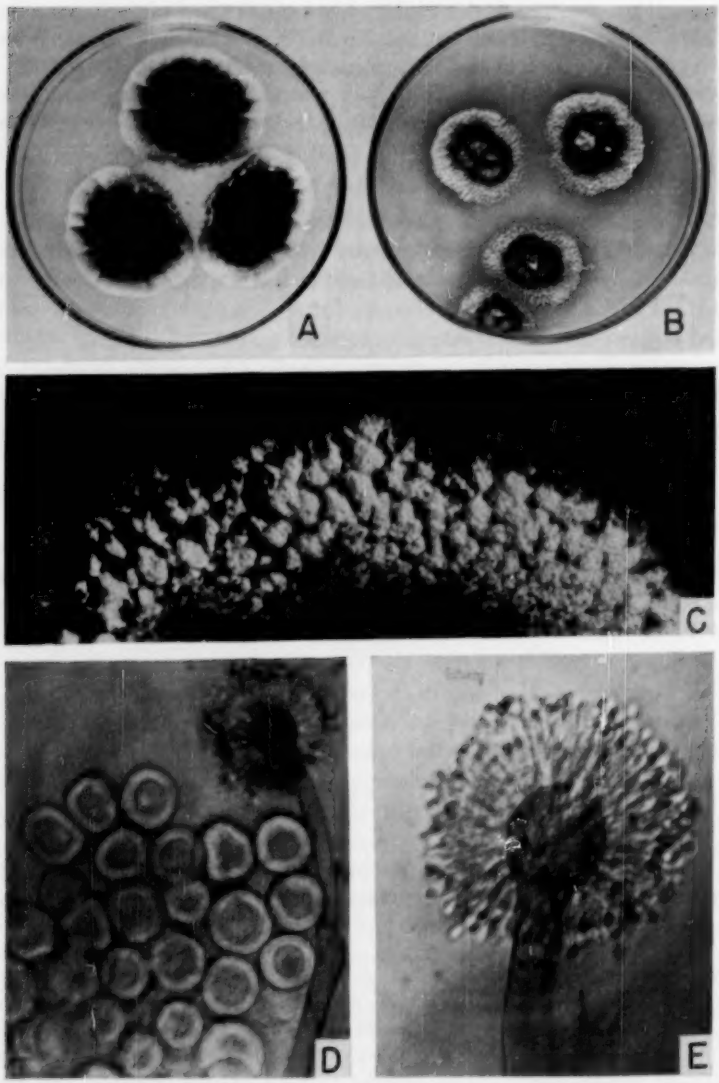

FIG. 7. *Aspergillus silvaticus;* NRRL 2398. *A* and *B*, Colonies on Czapek's solution and malt extract agars, respectively. *C*, Margin of colony on malt extract agar showing masses of hülle cells superficially suggesting perithecia and conidial structures arising through the hülle cell layer. *D*, Globose hülle cells, produced in abundance by this strain, × 365. *E*, Conidial head showing a nearly globose, pigmented vesicle bearing sterigmata in two series and globose to subglobose, rough conidia, × 700.

diam.; cellulae "hülle" perabundantes, globosae usque subglobosae, plerumque 15–25 μ in diam.

In culturis e solo, Africa occidentalis.

Colonies on Czapek's solution agar (FIG. 7A) growing well at room temperature (24° C), attaining a diameter of 4–5 cm in 10 days to 2 weeks, generally raised in central areas, radially furrowed, faintly zonate, with narrow growing margins white, shading quickly to Deep Colonial Buff (Ridgway, Pl. XXX) with the development of a nearly continuous layer of hülle cells; central colony area producing conidial structures in a dense stand, in deep forest-green shades near Dusky Yellow Green (Ridgway, Pl. XLI); reverse in buff to flesh-pink shades; exudate limited, colorless; odor rather pronounced, earthy. Conidial heads (FIG. 7E) very abundant, borne on conidiophores that arise through the hülle cell layer from submerged hyphae, radiate in pattern, mostly 200–300 μ in diameter, in rich yellow-green shades (see above); conidiophores mostly 250–270 μ in length, seldom exceeding 300 μ, 5.5–8.5 μ in diameter, with walls smooth and definitely brownish; vesicles nearly globose, colored as the conidiophores (FIG. 7E), mostly 14–17 μ in diameter but ranging from 11–19 μ, bearing sterigmata over nearly their entire surface; sterigmata in two series, crowded; primaries 5.5–7.0 $\times$ 2.8–3.3 μ; secondaries 5.5–7.0 $\times$ 2.2–2.8 μ; conidia globose to subglobose, deep yellow-green, conspicuously roughened, mostly 3.0–3.5 μ in diameter; hülle cells (FIG. 7D) very abundant, globose to subglobose, mostly 15–25 μ.

Colonies on malt extract agar (FIG. 7B) growing somewhat more restrictedly, 3.0–3.5 cm in 10 days to 2 weeks, basically as an Czapek's agar but less heavily sporing, with white growing margin less conspicuous and with hülle cells in the submarginal zone collected into definite clusters to produce a granular effect superficially suggesting a culture with abundant perithecia (FIG. 7C); reverse in dull orange-brown shades. Details of structural morphology as on Czapek's solution agar.

The binomial *A. silvaticus* was chosen because of the rich sylvan-green color of the massed conidial heads.

Type strain, NRRL *2398*, was isolated in May, 1950, from a sample of soil submitted by C. F. Charter, West African Cacao Research Institute, Tafo, Gold Coast, Africa.

The correct assignment of this beautiful species within the genus *Aspergillus* poses a difficult problem. The structural pattern and general dimensions of the conidial heads are strikingly suggestive of *A. sydowi* (Bain. & Sart.) Thom & Church. Pigmentation of the heads in the two species is, however, markedly different. Those of *A. sydowi* are bright *blue*-green when young, whereas those of *A. silvaticus* are in

rich *yellow*-green shades. Hülle cells are produced much more abundantly in this isolate than in any of the hundreds of strains of *A. sydowi* which we have examined, but are, however, of the same pattern as those occasionally seen in cultures of this species and in *A. versicolor*. They are, of course, produced abundantly in *A. janus*, Raper & Thom (**1944**), which is characterized by heads of two types, one type being indistinguishable from *A. sydowi*. On the other hand, globose hülle cells are regularly produced by species belonging to the *A. nidulans* group. Additionally, the brownish pigmentation of the conidiophores and vesicles in *A. silvaticus* is strikingly similar to the pigmentation of these structures in *A. nidulans* and allied species. Finally, hemispheric conidial heads of dark-green pigmentation are produced by *A. caespitosus*, a non-ascosporic member of the *A. nidulans* group. It is obvious that placement in either of the aforementioned groups will necessitate re-evaluation of the group characteristics established by Thom and Raper in their "Manual of the Aspergilli" (**1945**, FIG. 20), and must await a general revision of intrageneric relationships.

ASPERGILLUS TERREUS var. **africanus** var. nov.

A typo differt corporibus sclerotioideis globosis pallide alutaceis e cellulis separabilibus compositis.

Colonies on Czapek's solution agar (FIG. 8A) growing somewhat restrictedly, attaining a diameter of 3.0–3.5 cm in 10 days to 2 weeks at room temperature (24° C), consisting of a rather dense basal felt, irregularly buckled and wrinkled, in bright yellow shades near Barium Yellow to Citron Yellow (Ridgway, Pl. XVI), developing abundant conidial heads somewhat tardily and, like the species, eventually becoming light brown through cinnamon shades approaching Sayal Brown (Ridgway, Pl. XXIX). Conidial heads typical of the species in pattern and dimensions. Sclerotium-like bodies (FIG. 8C) produced in limited numbers, scarcely affecting the appearance of the colonies.

Colonies on malt extract agar spreading rapidly (FIG. 8B), attaining a diameter of 7–8 cm in 10 days to 2 weeks; growing margin thin, white, largely submerged, mature colony characterized by a dense layer of sclerotium-like bodies and interwoven mycelium, at first in bright yellow shades as on Czapek, approximating Deep Colonial Buff (Ridgway, Pl. XXX) at maturity. Conidial heads limited in number, not affecting colony appearance. Sclerotium-like bodies (FIG. 8C) in light tan shades when mature, globose or nearly so, variable in size but commonly 150–200 μ in diameter, consisting of a compact mass of irregularly swollen to rounded cells with walls more or less thickened, surrounded by a thin envelope of much-branched yellow mycelium, easily crushed, with

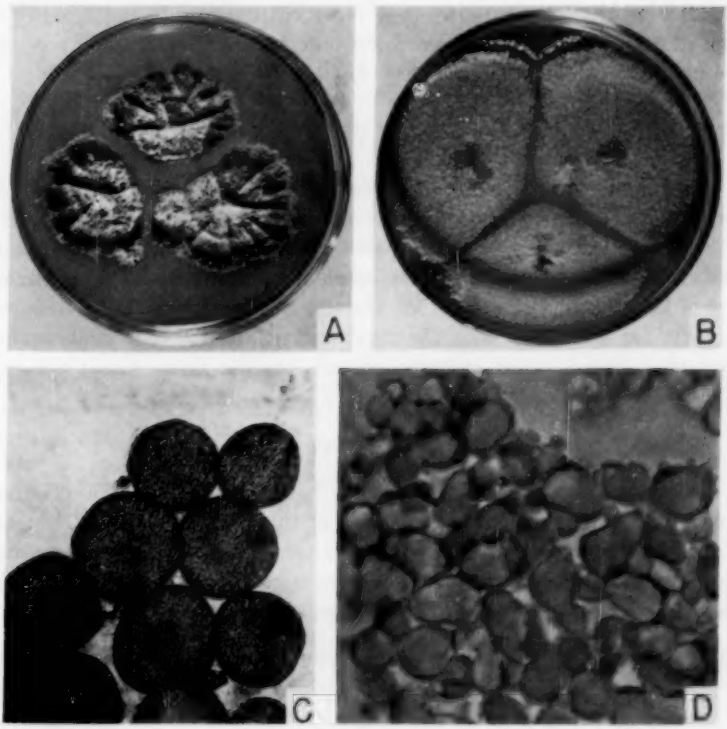

FIG. 8. *Aspergillus terreus* var. *africanus;* NRRL 2399. *A* and *B*, Colony patterns on Czapek's solution agar and malt extract agar, respectively. *C*, Sclerotium-like structures, produced in abundance on malt extract agar, × 55. *D*, Cells comprising the sclerotium-like bodies, demonstrating their tendency to separate, × 660.

constituent cells not adherent as in a true sclerotium but tending to separate readily (FIG. 8D).

Type strain, NRRL *2399*, isolated in May, 1950, from a sample of soil contributed by C. F. Charter, West African Cacao Research Institute, Tafo, Gold Coast, Africa.

The variety is distinguished particularly by the abundant sclerotium-like bodies produced on malt extract agar and other substrata commonly employed in our work. Superficially these bodies resemble true sclerotia. However, they are too easily crushed and the cells which comprise them are too readily separated one from another to permit their identification as such structures. They might appropriately be regarded as

pseudosclerotia. In any case, they represent the first instance in which formed bodies of any type have been observed in the *A. terreus* group. No evidence of an ascosporic stage has been seen.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to Miss Edith K. Cash, Plant Industry Station, Beltsville, and to Dr. Donald P. Rogers, N. Y. Botanical Garden, for preparing the Latin diagnoses; to photographers Eugene H. Herrling, Department of Plant Pathology, University of Wisconsin, and Roland W. Haines, Northern Utilization Research Branch, for their help with the illustrations; and to those collaborators who contributed either cultures or soil samples from which the new *Aspergilli* were isolated.

SUMMARY

A great many strains of *Aspergillus* have been isolated and examined since the publication of the "Manual of the Aspergilli" by Thom and Raper in 1945. Among this number, strains representing five new species and five new varieties have been encountered. The new species and varieties are described in the present paper, and include:

1. *Aspergillus paradoxus,* doubtfully a member of the *A. clavatus* group, characterized by small clavate vesicles and the production of yellow sclerotium-like bodies.
2. *Aspergillus aureolus,* an ascosporic member of the *A. fumigatus* group, characterized by vegetative mycelium in bright golden shades and ascospores with convex surfaces conspicuously echinulate.
3. *Aspergillus fischeri* var. *glaber,* duplicating the species in most particulars, but producing ascospores with convex surfaces smooth. (*A. fumigatus* group.)
4. *Aspergillus violaceus,* an ascosporic member of the *A. nidulans* series, characterized by a paucity of conidial structures and ascospores in deep violet-blue shades.
5. *Aspergillus nidulans* var. *acristatus,* duplicating the species generally but producing ascospores without equatorial crests or surface ornamentation.
6. *Aspergillus nidulans* var. *echinulatus,* duplicating the species in most particulars but producing ascospores with convex surfaces echinulate rather than smooth.

7. *Aspergillus variecolor* var. *astellatus,* duplicating the species generally, but showing lobed asci and producing ascospores with wide, non-stellate crests. (*A. nidulans* group.)
8. *Aspergillus deflectus,* a member of the *A. ustus* group, characterized by restrictedly growing colonies and conidial heads borne at or near right angles to the main axes of the conidiophores.
9. *Aspergillus silvaticus,* a non-ascosporic form which appears to be intermediate between the *A. versicolor* and *A. nidulans* groups.
10. *Aspergillus terreus* var. *africanus,* duplicating the species in most characteristics but producing abundant yellow sclerotium-like bodies.

The description of *Aspergillus unguis,* previously recognized as a non-ascosporic member of the *A. nidulans* group, is emended to include a strain which produces occasional perithecia and ascospores approximating those of *A. nidulans.*

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INDEX TO THE HELICOSPORAE

ROYALL T. MOORE

Linder (**8**), in 1929, published his monograph of the Helicosporae, which included all species known up to that time. Linder delimited the genera more precisely than had previous students of the group and this necessarily involved the rearrangement of many species in the genera as he delimited them. He also proposed a number of new species. In his treatment, however, he placed *Helicostilbe helicina*, the type species of *Helicostilbe* Höhnel, in synonymy with *Helicomyces scandens* Morgan, and designated *Helicostilbe simplex* Petch as the type of the emended genus. Since, according to the International Code of Nomenclature, a genus cannot be emended so as to exclude its type, *Helicostilbe* Linder is invalid. I therefore propose the new name *Trochophora* (from the Greek **Τροχός**, wheel and *φόρος*, bearer) to replace it.

Trochophora nom. nov.

Helicostilbe Linder, Missouri Bot. Gard. Ann. **16**: 333. 1929. Not *Helicostilbe* Höhnel 1902.

Type: *Helicostilbe simplex* Petch, Royal Bot. Gard. Peradeniya Ann. **7**: 321. 1922.

A new name must be given to the single species designated as belonging to the genus as so emended. The type species, therefore, becomes **Trochophora simplex** (Petch) comb. nov.

The main purpose of the present paper is to provide an index to the Helicosporae which will include all new genera and species published since 1929. It further attempts to correct certain errors that occur in Linder's keys, and in several cases the study of additional material has extended the quantitative limits of some species. In his original monograph (**8**) and his subsequent paper (**9**) on the genus *Helicoceras* Linder described 76 species distributed through 14 genera. This present paper brings the total to 95 species distributed through 17 genera.

It has been deemed advisable to exclude several taxa from the keys either because they are not clearly defined or because they do not appear significantly different from previously erected taxa. These taxa are appended to the respective keys as *inquirendae*. Also appended to the keys are those species which Linder (**8**) classed as "Species Imperfectly Known." Three other taxa are excluded: *Helicostilbe cantareirense*

Viegas (**16**) is excluded because its conidia, as illustrated, are not helicospores; *Helicomyces roseus* var. *major* Goidanich (**6**) is excluded because from the published description it does not appear to be sufficiently different from *roseus* to merit separate consideration; *Anulosporium nematogenum* Sherbakoff (**15**) is excluded because the hyphal nematode-snaring rings are probably not conidia (Sherbakoff was unable to germinate them), but the highly specialized traps which have been extensively described and illustrated by Drechsler in his papers on the zoopagaceous hyphomycetes. Arnaud (**1**) includes *Helicocephalum* in his treatment of the Helicosporae, but since it is generally agreed that this genus is quite close to the Mucorales, it is not included in the present paper.[1]

Also included in the present paper is the description of a new species of *Helicoma*.

Helicoma taenia sp. nov.

Conidiophoris aggregatis; conidiis fuscis, acropleurogenis, (7–)8–12(–16)-septatis, diametro spirarum 15.5–19.5 μ, filo 5.5–8 μ crasso, 1½–1¾ revolutiones faciente; conidiophoris ascendentibus, simplicibus aut tenuiter ramosis, ad 4 μ crassi, 600 μ tenus altitudine.

In cortice putrido *Populi*, Iowa City, Iowa, 3 Feb. 1942, GWM *5501* Typus. In herb. SUI.

Conidiophores aggregated; conidia fuscous, acropleurogenous, (7–)8–12(–16)-septate, 15.5–19.5 μ in diameter, filament 5.5–8 μ thick, coiled 1½–1¾ times; conidiophores ascending, simple or sparsely branching, about 4 μ thick by up to 600 μ long.

(Etym. ταινία—ribbon, in reference to the exceptionally long, slender conidiophores.)

This species is distinct on the basis of its very long, slender conidiophores which, when branched, usually divide once, a half to a third the distance from the base, and which arise in groups from a common area; the abundant conidia are produced singly on minute teeth. Assimulative mycelium is rarely seen. Although keying out adjacent to *H. recurvum*

[1] In a paper published after this study was submitted for publication, Arnaud (Soc. Myc. France Bull. **69**: 265–306. 1953) includes under the Helicosporae three new genera and six new species, as well as representatives of *Acrospeira*, all without Latin diagnoses. Many descriptions are too scanty (some are completely lacking!) to define even a genus, much less a new species. A case in point is *"Helicomyces ? elegans"* Arnaud, with the five word description "conidiophores simple et très courts." With one exception (*Diplorhynchus*, also a *nomen nudum* but included in the key), all of Arnaud's new Helicosporae are doubtful. Only further careful study will determine whether or not these invalidly published taxa represent distinct entities.

its conidial and conidiophore measurements as well as the unusual conidiophores of *recurvum* clearly distinguish the two species.

This work was done in the mycology laboratory of the State University of Iowa under the direction of Professor G. W. Martin.

KEY TO THE GENERA OF THE HELICOSPORAE

1. Conidia catenate, either barrel-shaped or in the form of a flattened helix *Helicodendron* Peyronel
1. Conidia not catenate 2
2. Conidia branched or furcate 3
2. Conidia simple, very rarely branched 4
3. Conidial filament thick in proportion to its length, tightly coiled, distal terminus bilobed so as to straddle the preceding part of the coil; with the single species *D. biloba* Arnaud nom. nud. ..*Diplorhynchus* Arnaud nom. nud. (1)
3. Conidia consisting of branched, septate coils, each coil loosely wound and comparatively thin in proportion to its length; with the single species *S. floriforme* Beverw. .....*Spirosphaera* Beverwijk (3)
4. Parasitic on vascular plants 5
4. Saprobic or doubtfully parasitic on other fungi 7
5. Conidia deeply constricted at the septa, appearing toruloid ..*Helicoceras* Linder
5. Conidia not constricted at the septa 6
6. Conidiophores aggregated into sporodochia *Drepanoconis* Schroeter & Hennings
6. Colonies appearing as dark fuzzy patches or sometimes effuse. Conidiophores 90–330 μ long; filaments straight to 1⅓ times coiled, 1–7-septate, pallid brown, 4.5–6.3 μ thick in the single species *H. caperoniae* Olive .....*Helicomina* Olive (13)
7. Conidiophores forming a loose, arachnoid, cottony or velvety colony, not compacted; or else apparently obsolete 8
7. Conidiophores aggregated to form a compact structure 12
8. Conidia coiled in three dimensions to form a cylindrical or barrel-shaped spore body .....*Helicoon* Morgan
8. Conidia coiled in two dimensions, or if in three, then not as above 9
9. Conidial filaments thick in proportion to their length, not hygroscopic 10
9. Conidial filaments thin in proportion to their length, hygroscopic 11
10. Conidia muriform .....*Xenosporella* Höhnel
10. Conidia transversely septate .....*Helicoma* Corda
11. Conidiophores and conidia hyaline; the conidia either borne on sessile teeth, or on conidiophores that are short erect branches arising from the creeping somatic mycelium .....*Helicomyces* Link
11. Conidiophores or conidia, or both, some shade of fuscous; conidiophores conspicuous .....*Helicosporium* Nees
12. Conidiophores aggregated to form a stele upon which the conidia are borne acrogenously. Synnemata scattered, up to 800 μ high by 30 μ thick, black; conidia solitary, fuliginous, 1–1¼ times coiled, 3–4-septate, 8–10 μ in diameter, filaments 4 μ thick in the single species *T. simplex* (Petch) Moore .....*Trochophora* Moore

12. Conidiophores aggregated to form pulvinate to irregularly globose, dry, horny, or gelatinous sporodochia 13
13. Conidia coiled in three dimensions to form a conical or oblong-ellipsoidal spore body. Filaments hyaline, about 7 μ thick, coiled 3–7 times to form a spore 40–45 × 18–22 μ in the single species *T. album* Harkn. *Troposporium* Harkness (**8**)
13. Conidia not coiled in three dimensions, or if so, then the filaments irregularly twisted and contorted 14
14. Conidiophores swollen at the apex below the conidia to form an apophysis-like structure; conidia coiled in two dimensions. Swellings of conidiophores 3–3.5 μ thick, 1.2–2.5 μ thick below; filaments hyaline, 2–4-septate, 1-coiled, 13–14 μ thick, spore diameter 17.5–21 (–25) μ in the single species *D. palmicola* Pat. ..*Delortia* Patouillard (**8**)
14. Conidiophores not apically swollen 15
15. Conidia twisted and contorted, coiled in three dimensions, filaments 7 μ or more thick; sporodochia large, gelatinous .....*Hobsonia* Berkeley
15. Conidia coiled uniformly in two dimensions, filaments less than 7 μ thick; sporodochia small 16
16. Conidiophores moniliform; sporodochia pulvinate to subspherical, the surface dry, granular to olivaceous. Filaments 9–13-septate, 1½–2 times coiled, 4–4.5 μ thick; coiled diameter 12–16 μ in the single species *T. fumosa* Kar. .....*Troposporella* Karsten (**8**)
16. Conidiophores slender, not moniliform; sporodochia wart-like, stipitate to substipitate .....*Everhartia* Saccardo & Ellis

GENERA DUBIA

1. ***Brachyhelicoon*** Arnaud nom. nud. Conidia spiriliform, aseptate, produced in heads containing 20–30 spores each, structure bearing the head simple, about 9 μ tall; with the single species *B. xylogenum* Arnaud nom. nud. (**1**).

2. ***Ophiodendron*** Arnaud nom. nud. Single species *O. Laocooni* Arnaud nom. nud. (**1**). This is the helical stage of Zalewski's complex 4-phase-4-spore *Clathrosphaera spirifera*. Linder (**8**), in discussing this complicated situation, states that this stage is probably *Helicodendron tubulosum* (Reiss) Linder. Since at present there is none of Zalewski's helical material available, but only the description in Zalewski's paper, it is, I believe, unwise, on such tenuous information, to accept Arnaud's name.

KEY TO THE SPECIES OF HELICODENDRON

1. Colonies white 2
1. Colonies at maturity some shade of fuscous 4
2. Filaments 1.2–1.75 μ thick, coiled in three dimensions to form short, 2–5 times coiled, cylindrical spore bodies 11 × 8–10 μ 1. *H. hyalinum* Linder (**8**)
2. Filaments 2.5–6.5 μ thick 3

3. Conidia slightly constricted at the septa, 1¼–2¾ times coiled to form flattened helices, 16–25.5 μ in diameter .....2. *H. triglitziensis* (Jaap) Linder (8)
3. Conidia not constricted at the septa, 4–12 times coiled to form cylindrical to ellipsoidal bodies, 18–22 × 20–32 μ .....3. *H. tubulosum* (Reiss) Linder (8)
4. Conidia loosely coiled in three dimensions, forming flattened helices ... 5
4. Conidia coiled in three dimensions, forming barrel-shaped spores 6
5. Conidia 30–50 μ in diameter, slightly constricted at the 1–3 septa, at first hyaline to light green, becoming darker with age, the filaments 5.5–6.5 μ thick, 1–3½ times coiled .....4. *H. paradoxum* Peyronel (8)
5. Conidia 15–25 μ in diameter, not constricted at the 1–9 septa, fuscous, filaments 4–4.5 μ thick, 2½ (1–7) times coiled. Aero-aquatic
5. *H. multicatenulatum* Beverwijk (3)
6. Conidia up to 200 × 70 μ, coiled to form a three dimensional helix of up to 20 coils. Aquatic .....6. *H. giganteum* Glen-Bott (5)
6. Conidia smaller, 14.5–40 × 10–19 μ 7
7. Filaments 1.5–2.5 μ thick, conidia 5–12 times coiled, 14.5–27 × 10.5–14.5 μ; conidiophores very short .....7. *H. fuscum* (B. & C.) Linder (8)
7. Filaments 3–4 μ thick, conidia 9–12 times coiled, 22–35(–40) × 15–19 μ; conidiophores 25–50 μ long. Aero-aquatic ...8. *H. westerdijkae* Beverwijk (3)

KEY TO THE SPECIES OF HELICOCERAS (9)

1. Conidia smooth, cells shorter than wide; conidiophores not conspicuously inflated nor densely branched at the apices 2
1. Conidia echinulate, cells longer than wide; conidiophores mostly inflated at the apex and often densely short-branched 3
2. Conidia 5–7.5(–9) μ thick. On *Celtis* spp.
1. *H. Celtidis* (Biv.-Bernh.) Linder
2. Conidia 8–13 μ thick. On *Plantago* spp.
2. *H. Plantaginis* (Cda.) Linder
3. Conidia 60–90 × 5–18 μ. On *Nymphaea* spp.
3. *H. Nymphaearum* (Rand) Linder
3. Conidia 64–90 × 5.4–9 μ. On seeds of *Oryza* ....4. *H. Oryzae* Linder & Tullis

KEY TO THE SPECIES OF DREPANOCONIS (8)

Conidiophores 30 × 2–3 μ; conidia hyaline, ½–1-coiled, 4–9-celled, 16–25 μ in diameter, filaments 10–17 μ thick .....1. *D. larvaeformis* Spegazzini
Conidiophores absent; conidia subsessile, hyaline, 1–1½-coiled, 5–13-septate, 21–41.5 μ in diameter, filaments 9–18 μ thick
2. *D. anguisporus* (Pat. & Lagerh.) Linder

KEY TO THE SPECIES OF HELICOON

1. Conidiophores of colonies on natural substrate up to 20 μ long (of one species may reach up to 50 μ when in culture) 2
1. Conidiophores exceeding 20 μ, generally much longer 4
2. Conidia fuscous, 50–60 × 50–80 μ; conidiophores 1–1.5 μ long
1. *H. Richonis* (Boudier) Linder (8)
2. Conidia hyaline, smaller, 20–36 × 25–60 μ 3

3. Conidia ellipsoidal, 20–30 × 37–56 μ, filaments 4.5–6.5 μ thick, coiled 5–16 times; conidiophores apparently obsolete on natural substratum, seldom exceeding 50 μ in culture .....2. *H. sessile* Morgan (8)
3. Conidia short, almost ovoid, 25–27 × 23–36 μ, filaments 3.3–4.5 μ thick, coiled 5–8 times; conidiophores 15–20 μ long .....3. *H. farinosum* Linder (8)
4. Conidiophores simple or little branched 5
4. Conidiophores much branched, anastomosing, up to 800 μ long 8
5. Conidia golden-yellow, 8–16 times coiled, 19.5–27 × 36–45 μ; conidiophores 32–150 μ long .....4. *H. auratum* (Ellis) Morgan (8)
5. Conidia hyaline to fuscous, 3–9 times coiled 6
6. Filaments prominently constricted at the septa; conidia (3–)4(–5) times coiled, dilute fuscous to brown with age, (14.5–)19.8(–22) × (23.5–)30.5–36(–41.5) μ; conidiophores 90–230 μ long
5. *H. Thaxteri* Linder (11)
6. Filaments not constricted at the septa 7
7. Filaments coiled to form subovate to elliptical spore bodies, 20–25 × 22.5–33 μ long, light fuscous at maturity, 6–9 times coiled; conidiophores 70–200 μ long .....6. *H. fuscosporum* Linder (8)
7. Filaments coiled to form blunt, oval spore bodies 31–45 × 23–30 μ long, dark fuscous at maturity, (4–)5–6(–7) times coiled; conidiophores 25–320 μ long. Aero-aquatic .....7. *H. pluriseptatum* Beverwijk (4)
8. Conidia 18–27 × 28–45 μ, 7–10 times coiled, filaments 4.5–5.5 μ thick
8. *H. ellipticum* (Peck) Morgan (8)
8. Conidia 16–18 × 18–24 μ, 6–8 times coiled, filaments 2.3–3.6 μ thick. Doubtfully distinct from *ellipticum* .....9. *H. reticulatum* Linder (8) [2]

Species imperfectly known: *Helicoon politulum* (Schulzer) Lindau.

KEY TO THE SPECIES OF XENOSPORELLA (8)

1. Conidia 38–55 μ in diameter, deep fuscous to opaque and black. Perfect stage *Acanthostigmella Thaxteri* Linder (8) .....1. *X. Thaxteri* Linder
1. Conidia less than 35 μ in diameter 2
2. Mature conidia subhyaline to dilute fuscous, 15.5–20 μ in diameter, the filament not, or rarely, coiled around a central cell
2. *X. larvalis* (Morgan) Linder
2. Mature conidia fuscous to deep fuscous, the filament coiled around a central cell 3
3. Conidiophores hyaline to subhyaline, or, if fuscous, only at the base; filaments 12.5–17 μ thick, conidia 24–30 μ in diameter ..3. *X. pleurococca* Höhnel
3. Conidiophores deep fuscous; filaments 9.5–11.5 μ thick, conidia 23–27 μ in diameter .....4. *X. Berkeleyi* (Curtis) Linder

[2] Linder (8) says that "This species closely resembles *Helicoon ellipticum* in the appearance of the colonies and conidiophores. The smaller spores, however, separate it from that species. The range is apparently more southern." However, collections of *ellipticum* have been studied that have spores extending over the total range. Further, although the type and one other collection of *reticulatum* are from Mississippi and Alabama respectively, Linder also cites three collections from New England, thus indicating that the ranges of the two species overlap, at least in part. It is quite possible, therefore, that the distinction between *ellipticum* and *reticulatum* is environmental rather than specific.

KEY TO THE SPECIES OF HELICOMA

1. Fungi occurring in brackish or salt water 2
1. Fungi occurring in terrestrial habitats 3
2. Conidia 4–10 septate, 1¾–2 times coiled, coil diameter 23–26.5 μ, filaments 8.5–10 μ thick .....1. *H. maritimum* Linder (2)
2. Conidia 11–26-septate, 1¾–4½ times coiled, coil diameter 28–39 μ, filaments 5.5–8 μ thick, tapering to 3.5–4.5 μ near the rounded truncate bases .....2. *H. salinum* Linder (2)
3. Conidial diameter 51–72 μ, filaments 28–31 μ thick, 6–9-septate 3. *H. roseolum* Thaxter (8)
3. Conidia smaller, less than 45 μ in diameter 4
4. Conidial diameter 4–7 μ, filaments 1 μ thick, hyaline 4. *H. minutissimum* Linder (8)
4. Conidial diameter greater than 7 μ, filaments more than 1 μ thick 5
5. Conidiophores commonly anastomosing, sparsely branched, ascending, (20–)30–60(–100) × 5.5(–6.5) μ; conidia generally acrogenous, obliquely attached to conspicuous, slender, cylindrical, sporogenous teeth, coil diameter 19.5–23.5 μ, filaments hyaline, 1½–1¾ times coiled, 3.5–4 μ thick, 18–25-septate .....5. *H. anastomosans* Linder (10)
5. Conidiophores very rarely anastomosing 6
6. Conidiophores characterized by the presence of lateral swellings on which conidia were previously produced, the tip inflated and bearing conidia singly, swellings resulting from continued conidiophore growth; conidial diameter 9–12 μ, filaments 5–6 μ thick, 3–4-septate 6. *H. simplex* (Sydow) Linder (8)
6. Conidiophores, if present, characterized by the presence of distinct teeth on which the conidia are or were previously produced, or else conidia sessile 7
7. Mycelium when growing on the surface of the substratum conspicuously inflated between the septa; the terminal or subterminal cells of the conidiophores denticulate, frequently inflated and 8.5–12 μ thick at the widest part or else not inflated and then rounded-tapering at the apices; conidia 13.5–18 μ in diameter, 1¼–1¾ times coiled, hyaline; filaments (3–)4–7(–8)-septate, (4–)5–6(–7) μ thick .....7. *H. inflatum* Linder (11)
7. Mycelium and conidiophores not conspicuously inflated 8
8. Conidial diameter 7–10 μ, filaments hyaline, 1–3-septate, ¾–1½ times coiled, 3–3.5 μ thick, occurring on Sphaeropsidaceae 8. *H. stigmateum* (Reiss) Linder (8)
8. Conidial diameter greater than 10 μ, septa and coils more numerous ... 9
9. Conidiophores only very rarely more than 60 μ long in mature colonies, generally shorter 10
9. Conidiophores longer than 60 μ common in mature colonies, although shorter ones may be present 16
10. Filaments 2–2.5 μ thick, 12–20-septate, coiled 2–2¾ times, conidial diameter 11–12.5 μ; conidiophores up to 30 μ long 9. *H. microscopicum* (Ellis) Linder (8)
10. Filaments thicker, 2.5–12 μ 11

11. Conidia tapering to the narrow or truncate basal ends 12
11. Conidia abruptly rounded at the basal ends 13
12. Conidia coiled 1½–1¾ times, coil diameter 16–20 μ, filaments 4.5–5.5 μ thick, tapering to 1–1.5 μ at the base, 8–10(–15)-septate, hyaline, maximum length of the conidiophores about 20 μ
10. *H. polysporum* Morgan (8)
12. Conidia coiled 2–3 times, coil diameter 18–21 μ, filaments about 4 μ thick, tapering to the apiculate basal end, hyaline, multiguttulate; conidiophores as very short, about 20 μ, ascending branches covered by abundant spores .....11. *H. repens* Morgan (8)
13. Conidia 20–30 μ in diameter, filaments 15–25-septate 14
13. Conidia 11–19 μ in diameter, filaments 7–15-septate 15
14. Conidiophores branched to much branched, 25–60(–80) μ long; filaments 2.5–3.5 μ thick, 18–25-septate, 2¾–3¼ times coiled, conidial diameter 21–28 μ .....12. *H. tenuifilum* Linder (10)
14. Conidiophores simple, about 25 μ long, filaments about 4 μ thick, 15–20-septate, 2–3 times coiled, conidial diameter 20–25 μ
13. *H. limpidum* Morgan (8)
15. Conidia coiled 2–2¼ times, (12–)14.4–19 μ in diameter, filaments (7–)10–15-septate, 3.5–5 μ thick; conidiophores up to 55 μ long; colonies minute tufts less than 1 mm in diameter .....14. *H. monilipes* Ellis & Johnson (8)
15. Conidia coiled 1½–1¾ times, 11.5–15.5 μ in diameter, filaments 3–12-septate, 3.5–5.5 μ thick; conidiophores 10–30 μ long; colonies as above
15. *H. olivaceum* (Karsten) Linder (8)
16. Conidia 16–30-septate, 2–3 times coiled 17
16. Conidia 3–16-septate, 1–2 times coiled 19
17. Conidial diameter 36–44 μ, filaments fuscous, 2½–3 times coiled, 7.5–9 μ thick, 16–18-septate, conidiophores 40–150 μ long
16. *H. perelegans* Thaxter (8)
17. Conidial diameter 21–27 μ; filaments hyaline 18
18. Conidia on stout teeth, 2–3 μ thick; conidiophores 22–150 μ long; filaments 4–4.5 μ thick, 16–24-septate, conidial diameter about 26 μ
17. *H. violaceum* Winter (8)
18. Conidia on slender teeth, 1.2–2 μ thick; conidiophores 27–250 μ long, filaments 3.5–4.5(–5.5) μ thick, 18–30-septate; coiled diameter 21–27 μ .....18. *H. Morgani* Linder (8)
19. Conidia not constricted at the septa 20
19. Conidia constricted at the septa 32
20. Conidia in mature colonies acrogenous 21
20. Conidia in mature colonies pleurogenous or acropleurogenous 24
21. Conidia 10–16-septate 22
21. Conidia 3–9-septate 23
22. Conidia 14–16-septate, 30–35 μ in diameter, filaments 8 μ thick; colonies effuse, small or confluent, velvety, black; conidiophores erect, rather stout, filiform, 130–160 μ long
19. *H. intermedium* (Penzig & Saccardo) Linder (8)
22. Conidia 10–12-septate, 36–40 μ in diameter, filaments 11–12 μ thick; colonies and conidiophores as above ....20. *H. palmigenum* Linder (8)

23. Conidiophores up to 200 μ long by 5.4–7.2 μ thick; conidial diameter 14.4–18(–20) μ, filaments 4–9-septate, once coiled, 4.5–5.4(–7.5) μ thick. Perfect stage *Lasiosphaeria pezizula* (B. & C.) Saccardo (8)
21. *H. Curtisii* Berkeley (8)

23. Conidiophores 60–245 × 2.8–3.5 μ; conidial diameter 12.5–14.5 μ, filaments 3–5-septate, once coiled, 7.2μ thick ..22. *H. fasciculatum* Berkeley & Curtis (8)

24. Conidia 30–40 μ in diameter, fuscous, 10–16-septate 25

24. Conidia 10–25 μ in diameter, hyaline, to dilute fuscous, or yellow ochraceous, 3–12(–16)-septate 26

25. Colonies effuse, forming a dark brown, hirsute layer; filaments 8–11 μ thick, 1¼–2 times coiled, 10–16-septate, coiled diameter 30–35 μ, borne on stout teeth; conidiophores 150–310 μ long .....23. *H. atroseptatum* Linder (8)

25. Colonies inconspicuous, of scattered conidiophores; filaments 11.5–13.5 μ thick, 1½–1¾ times coiled, 11–14-septate, coiled diameter 33.5–38 μ, sessile, provided with a distinct hyaline upward-tapering collar at the base of the filament; conidiophores 171–216(–252) μ long ...24. *H. Westoni* Linder (10)

26. Conidia with truncate bases, borne on stout (1.5–5 μ thick) teeth 27

26. Bases of conidia tapering to an apiculate or tapering-rounded end, sporogenous teeth less than 1.5 μ thick 28

27. Sporogenous teeth 1.5–2.5 μ thick, seldom more than once-branched; conidial diameter (14–)16–19 μ, filaments 3.5–5.5 μ thick, 1½–1¾ times coiled, (5–)7–9(–11)-septate; conidiophores 45–150 μ long
25. *H. Muelleri* Corda (8)

27. Sporogenous teeth 2–5 μ thick, at maturity more than once-branched; conidial diameter 17–24 μ, filaments 5–6.5(–7) μ thick, 1¼–2 times coiled, inconspicuously 3–7(–11)-septate; conidiophores 75–175 μ long. Pediceled sclerotia present .....26. *H. proliferens* Linder (8)

28. Conidiophores ascending or repent, much branched, less than 6 μ thick, up to 200 μ long; base of conidia abruptly rounded and attached obliquely to the conidiophore; conidial diameter 18–20 μ, filaments 1½–1¾ times coiled, 6–8-septate, 5.4–6.5 μ thick
27. *H. ambiens* Morgan (8)

28. Conidiophores erect or bent, sparsely branched, or, if much branched, then more than 6 μ thick, and conidia not abruptly rounded at the base 29

29. Conidiophores erect, conspicuously branched, 55–250 × 5.5–7.5 μ, enveloped in part with a rough crystal sheath; conidial diameter at maturity 15–18 μ, filaments 1½–1¾ times coiled, 8–10-septate, 4.5–6.5 μ thick
28. *H. asperothecum* Linder (8)

29. Conidiophores not conspicuously branched, not enveloped by a rough crystal sheath, although crystal deposits may be present 30

30. Conidiophores hyaline, 5.5 μ thick, at maturity about 180 μ long; conidia hyaline to dilute yellowish ochraceous, 14–17.5 μ in diameter, filaments 1¼–1¾ times coiled, 5–7-septate, 5–5.5 μ thick
29. *H. conicodentatum* Linder (8)

30. Conidiophores fuscous 31

31. Conidiophores 6–8 μ thick, up to 200 μ long, the terminal cells occasionally constricted at septa, generally simple, producing scattered masses of pur-

plish-black crystalloid deposits; conidia hyaline to dilute fuscous, acropleurogenous, (3–)6–8-septate, 15–17(–20) μ in diameter, filaments 6–8 (–10) μ thick, coiled 1–1¾ times .....30. *H. recurvum* (Petch) Linder (**8**)

31. Conidiophores about 4 μ thick, up to 600 μ long, the terminal cells not constricted at septa, simple or sparsely branched, without crystalloid masses; conidia pale fuscous, profusely acropleurogenous, (7–)8–12(–16)-septate, 15.5–19.5 μ in diameter, filaments 5.5–8 μ thick, coiled 1½–1¾ times
31. *H. taenia* Moore

32. Filaments 4.5–5.5 μ thick, 8–12-septate, 1½–2 times coiled, conidial diameter 12.5–20 μ; conidiophores 80–115 μ long
32. *H. phaeosporium* Fresenius (**8**)

32 Filaments 5.4–8 μ thick, 10–14-septate, 1½–2 times coiled, conidial diameter 18–25 μ; conidiophores 110–125 μ long
33. *H. velutinum* Ellis (**8**)

Species imperfectly known: *Helicoma candidum* (Pr.) Linder.

KEY TO THE SPECIES OF HELICOMYCES

1. Bristle-like setae present in older colonies; hyphae creeping over the substratum or climbing the erect bristle-like setae and forming conidial clusters; filaments 3–4 times coiled, 1.5–3 μ thick ....1. *H. scandens* Morgan (**8**)
1. Colonies without bristle-like setae 2
2. Colonies ceraceous, when young the whole stratum composed of semi-agglutinated mycelium, conidia, and conidiophores, when old, crust-like and composed almost entirely of conidia; filaments tapering from 8 μ thick in the middle to about 3.5 μ thick at the ends, brittle, each cell containing 1 or 2 vacuoles, coiled diameter (32–)50–65 μ
2. *H. colligatus* Moore (**12**)
2. Colonies flocculose, not ceraceous 3
3. Filaments 5–7 μ thick, the basal cell truncate, coiled in three dimensions to form a flattened helix 30–40 μ in diameter
3. *H. ambiguus* (Morgan) Linder (**8**)
3. Conidial filaments 1.5–5 μ thick, coiled in two dimensions to form a flat disk .. 4
4. Sterile mycelium and conidiophores fuscous or dilute fuscous 5
4. Sterile mycelium and conidiophores mostly hyaline 6
5. Conidia pleurogenous, borne on minute lateral teeth from the repent creeping mycelium, filaments 2.5–3 μ thick, 2½–3½ times coiled, conidial diameter about 35 μ .....4. *H. bellus* Morgan (**8**)
5. Conidia mostly acrogenous, borne on stout teeth from conidiophores 1–3 (–4)-septate, 18–39(–50–58) × 2.5–3.6(–4) μ, that for the most part arise directly from the substratum, filaments 3.5–4.5 μ thick, 1¼–2¼(–2½) times coiled, conidial diameter (23–)39.5–62 μ ....5. *H. fuscopes* Linder (**10**)
6. Conidial diameter 80–120 μ, filaments 1.5–2 μ thick; colonies arachnoid or effuse, suborbicular, 5–15 mm or more in diameter
6. *H. tenuis* Spegazzini (**8**)
6. Conidial diameter 30–45 μ, filaments 2.5–4.5(–5.5) μ thick; colonies effuse, forming a thin flocculose white to pinkish layer .....7. *H. roseus* Link (**8**)

Species imperfectly known: *Helicomyces albus* Preuss.

KEY TO THE SPECIES OF HELICOSPORIUM

1. Conidia yellow or greenish-yellow in mass 2
1. Conidia in mass some other color 5
2. Conidiophores dilute fuscous to subhyaline, pellucid, arising from repent or climbing mycelium; conidia borne singly on minute, hyaline teeth on the creeping, fertile mycelium or on the lower portions of the erect conidiophores, filaments 1–1.5 μ thick, coiled diameter 10–15 μ .....1. *H. gracile* (Morgan) Linder (8)
2. Conidiophores fuscous or deep fuscous, at first simple or erect 3
3. Conidia borne on minute cylindrical teeth or slender branches, filaments 1 μ thick, coiled diameter 10–15 μ .....2. *H. vegetum* Nees (8)
3. Conidia borne on hyaline, bladder-like lateral projections from the conidiophores 4
4. Conidiophores branching above at maturity, 5.4–7.2 μ thick near the base, 390–650 μ long; conidial diameter 16–19 μ, filaments 1–2 μ thick .....3. *H. aureum* (Corda) Linder (8)
4. Conidiophores not branching above at maturity, 3.5–4.5 μ thick near the base, up to 480 μ long; conidial diameter about 21.6 μ when dry, filaments 1.4–1.6 μ thick .....4. *H. guianensis* Linder (8)
5. Filaments generally more than 6 μ thick 6
5. Filaments generally less than 6 μ thick 7
6. Conidia 3–5-septate, 50–90 μ in diameter, acrogenous, filaments 6–10 μ thick .....5. *H. insuetum* Petrak (14)
6. Conidia multiseptate 15
7. Filaments 3–6 μ thick 8
7. Filaments less than 3 μ thick, generally not exceeding 2.5 μ 9
8. Conidia borne on stout teeth 2.5–5 × 1.6–2(–3.1) μ; filaments 3–5 μ thick; conidiophores up to 275 μ long. When cultured on rolled-oat medium, spores in mass in old cultures becoming deep maroon (Hessian Brown of Ridgway) .....6. *H. Linderi* Moore (12)
8. Conidia borne on stout, shorter teeth 1.8–3.6 × 1.8 μ; filaments 4.5–6 μ thick; conidiophores 90–250 μ long. Perfect stage *Lasiosphaeria nematospora* Linder (8) .....7. *H. nematosporum* Linder (8) [3]
9. Conidiophores sparsely branched, or, if branched, then not anastomosing at frequent intervals 10
9. Conidiophores much branched and anastomosing at frequent intervals 11
10. Conidiophores short, 40–70 μ long at maturity; filaments 2.5–4.5 μ thick, conidial diameter 20–30 μ .....8. *H. panacheum* Moore (12)
10. Conidiophores at maturity very much longer 12
11. Colonies velvety, up to 400 μ thick, easily separable from the substratum as loose mats of conidiophores; conidial diameter 18–25 μ, filaments 0.9–2 μ thick .....9. *H. lumbricoides* Saccardo (8)

[3] Hughes (Can. Jour. Bot. **31**: 608. 1953) in a footnote states: ". . . three species included in *Helicosporium* by Linder are considered best classified in the apparently dry spored genus *Drepanospora* [*Drepanispora*] Berk. & Curt. e.g. *D. pannosa* Berk. & Curt. (= *Helicosporium serpentinum* Linder), and the *Drepanospora* conidia of *Lasiosphaeria nematospora* Linder [*H. nematosporum* Linder] and *L. elinorae* Linder [*H. Elinorae* Linder]."

11. Colonies effuse, short-velvety, up to 200 μ thick, not easily separable from the substratum; conidial diameter 21–29 μ, filaments 1.5–2.5 μ thick. Doubtfully distinct from *lumbricoides* .....10. *H. lumbricopsis* Linder (**8**) [4]
    12. Conidia 6–9 μ in diameter, filaments 0.75–1.5 μ thick; conidiophores fuscous, at first simple, erect, becoming branched and decumbent and bearing conidia on bladder-like projections
        11. *H. decumbens* Linder (**8**)
    12. Conidia 10–12 μ in diameter, not borne on bladder-like projections; conidiophores subhyaline to dilute fuscous, elongate, slender 13
13. Conidial filaments 1.5–2.5 μ thick, coil 15–20 μ in diameter; conidiophores branching below. Perfect stage *Tubeufia helicomyces* Höhnel (= *Acanthostigmella genuflexa* Höhnel?; see culture studies, Webster (**17**), and studies of type material, Linder (**8**). See also discussion of *H. albidum* below.) .....12 *H. phragmitis* Höhnel (8)
13. Filaments 1 μ thick, conidial diameter 12.5–15 μ; conidiophores sparsely branching below 14
    14. Conidiophores clearly septate, mostly simple, not anastomosing above, 108–250 × 3.5–4.5 μ; conidia 12.5–14.5 μ in diameter
        13. *H. griseum* (Bon.) Saccardo (**8**)
    14. Conidiophores indistinctly septate, elongate, slender, sparsely anastomosing above, up to 580 μ long by 1.5–4 μ thick; conidial diameter 10–15 μ .....14. *H. pallidum* Cesati (**8**)
15. Filaments 6–8 μ thick, borne pleurogenously on stout teeth, 4 × 5–7 μ; terminal cells of conidiophores granulate-roughened by crystal-like deposits. Perfect stage *Lasiosphaeria Elinorae* (**8**) .....15. *H. Elinorae* Linder (**8**) [5]
15. Filaments 8–10 μ thick 16
    16. Epispore smooth; filaments flexuous throughout, 8–10 μ thick, up to about 300 μ long, conidial loosely coiled, 55–100 μ in diameter
        16. *H. serpentinum* Linder (**8**) [3]
    16. Epispore reticulate; basal portion of mature filaments straight, distal portion tightly coiled, up to as many as 10 revolutions and 30 μ in diameter, 8 μ thick by about 800 μ long
        17. *H. Hendrickxii* Hansford (**7**) [5]

Helicosporium Hendrickxii Hansford

Plagulae amphigeneae effusae tenues brunneae. Mycelium ex hyphis dilute fuligineis 1.5–2.5 μ crassis laxe irregulariterque reticulatis compositum, etiam paucis 4 μ crassis fuscis, levis vel distincte areolato-muriculatis. Conidia ex ramis erectis hypharum mycelii efformantur, cylindracea fusca, usque ad 800 × 8 μ, septata (cellulis

[4] This species is recorded by Linder (**8**) as being tropical or subtropical, but recently identified collections from Iowa City, Iowa, considerably extend this range. It may well be that the differences between *lumbricoides* and *lumbricopsis* are environmental rather than specific.

[5] I wish to express my deep appreciation to Dr. E. P. Wiltshire, Director of the Commonwealth Mycological Institute, Kew, England, to Dr. R. Vanbreuseghem, Institut de Medecine Tropicale Prince Leopold, Anvers, Belgium, and to Dr. C. G. Hansford, University of Adelaide, Adelaide, S. Australia, for their assistance in letting me see the original description and illustration of this fungus. The following is the original Latin diagnosis:

15–20 μ longis) saepe leniter constricta, episporio hexagonaliter areolato vel muriculato.

Hab. in foliis emortuis *Cyrtostachydis Rendlei*, Yangambi, Congo Belge, *Hendrickx 2360 Myc.*

Species imperfectly known: *Helicosporium albo-carneum* (Cr.) Saccardo, *H. brunneum* Schulzer & Saccardo, *H. Ellisii* Cooke, *H. herbarum* Sacc., Bomm. & Rouss, *H. populi* (Cr.) Saccardo, *H. prasinum* Preuss, *H. pulvinatum* (Nees) Persoon.

SPECIES INQUIRENDAE

Helicosporium albidum Grove, Jour. Bot. **24**: 204. 1886. This species differs from the later described *H. phragmitis* only in having slightly smaller conidia, but material of *albidum* is unavailable and the description is inadequate. However, *phragmitis* has been studied from type material by Linder, and it has also been obtained in culture by Webster from ascospores of *Tubeufia helicomyces*. Linder maintained the two species because he was convinced that *Acanthostigmella genuflexa* on the type material was the perfect stage as had been reported by Höhnel. Whether *A. genuflexa* is synonymous with *T. helicomyces* or whether it has any relation at all to *H. phragmitis* is still an open question.

KEY TO THE SPECIES OF HOBSONIA (8)

Filaments with conspicuous vacuoles in each cell, freely proliferating, (6–)8–12(–14) μ thick, hyaline, multiseptate; conidiophores slender, 1.8–3 μ thick; sporodochia yellowish when dry. Tropical .....1. *H. gigaspora* Berkeley

Filaments with denser protoplasm, vacuoles rare, sparsely proliferating, (9–)12–15(–17) μ thick, hyaline, multiseptate, conidiophores slender, 1.5–3 μ thick; sporodochia yellowish when dry. Temperate. Doubtfully distinct from *gigaspora* .....2. *H. mirabilis* (Peck) Linder

KEY TO THE SPECIES OF EVERHARTIA (8)

1. Conidia 3-septate, (7.5–)8–10(–13?) μ in diameter, 1-convolute, filaments (3–)3.5–4 μ thick; sporodochia yellowish, stipitate or substipitate, (195–)250–400 × (90–)100–150 μ .....1. *E. lignitalis* Thaxter

1. Conidia more than 3-septate 2

2. Conidia 4–8-septate, 9–12 μ in diameter, 1½–1¾ times coiled, filaments 3–3.5 μ thick; sporodochia white to yellowish, irregularly hemispherical or elongate, 0.5–1 × 0.5–2 mm ....2. *E. candida* Thaxter

2. Conidia 16–25-septate, 13–18(–20) μ in diameter, 2–2½ times coiled, filaments 2.5–3.5 μ thick; sporodochia dark fuscous, sessile, small 3. *E. hymenuloides* Saccardo & Ellis

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A NEW SPECIES OF ELSINOË ON SOUTHERN MAGNOLIA [1]

JULIAN H. MILLER AND ANNA E. JENKINS [2]

(WITH 5 FIGURES)

In describing *Sphaceloma magnoliae* on southern magnolia the writers (**3**) mentioned the associated immature perfect stage, recognizable as *Elsinoë*. Mature ascocarps now available make possible the diagnosis of the fungus as of this genus.

Elsinoë magnoliae sp. nov. FIGS. 1–5.

Sphaceloma magnoliae Jenkins & Miller,
Jour. Wash. Acad. Sci. **42**: 323, f. 1, 1952.

Maculae epiphyllae, sparsae vel aggregatae et coalescentes, saepe per nervum contractae vel areas laminae marginales vel apicicales involventes, circulares vel angulares, subelevatae, albae usque griseae, brunneo-marginatae, usque 1.5 mm in diam., stromata ascogena vel conidifera in massis atris papillosis vel applanatis, solitariis vel dispersis gerentes; ascomata subcuticularia vel intraeidermicalia, superficie superiori denudata, subspherica usque applanata, 30–240 μ in diam., 20–40 μ crassa, ex parte inferiori et inter ascos e cellulis minutis hyalinis parenchymaticis, supra e strato cellulorum fuscorum crassitunicatorum epithecium 10–15 μ crassum efficienti composita; asci numerosi in stratis 1–2 irregulariter dispersi, globosi usque late elliptici, sessiles, tunica interiori praecique ad apicem incrassata, 17.5–30 × 14–20 μ; ascosporae irregulariter dispositae, clavato-ellipoideae, 3-septatae, interdum longitudinaliter divisae, 9–14 × 3.5–6 μ; stromata conidifera sporodochioidea vel acervularia: sporodochia e stromate pallido pseudoparenchymatico subcuticulari oriunda; conidiophora e cellulis fuscis crasso-tunicatis in superficie stromatis oriunda, divergentia, recta vel geniculata, usque 4-septata, fusca, ad apices pallidioria, 20–45 × 4–5 μ; conidia acrogena, apiculis brevibus producta, unicellularia, ellipsoidea, hyalina, 6–12 × 3–5 μ; acervuli intraepidermicali-

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[2] Julian E. Miller is chairman of the Division of Plant Pathology and Plant Breeding, College of Agriculture, University of Georgia; Anna E. Jenkins, Mycologist, Foreign Operations Administration. They are indebted to Dr. A. A. Bitancourt, Director, Divisão de Biologia Vegetal, Instituto Biológico, São Paulo, Brazil, for his critical reading of this article in manuscript. They are also pleased to acknowledge the assistance of Miss Edith K. Cash in translating the diagnosis into Latin.

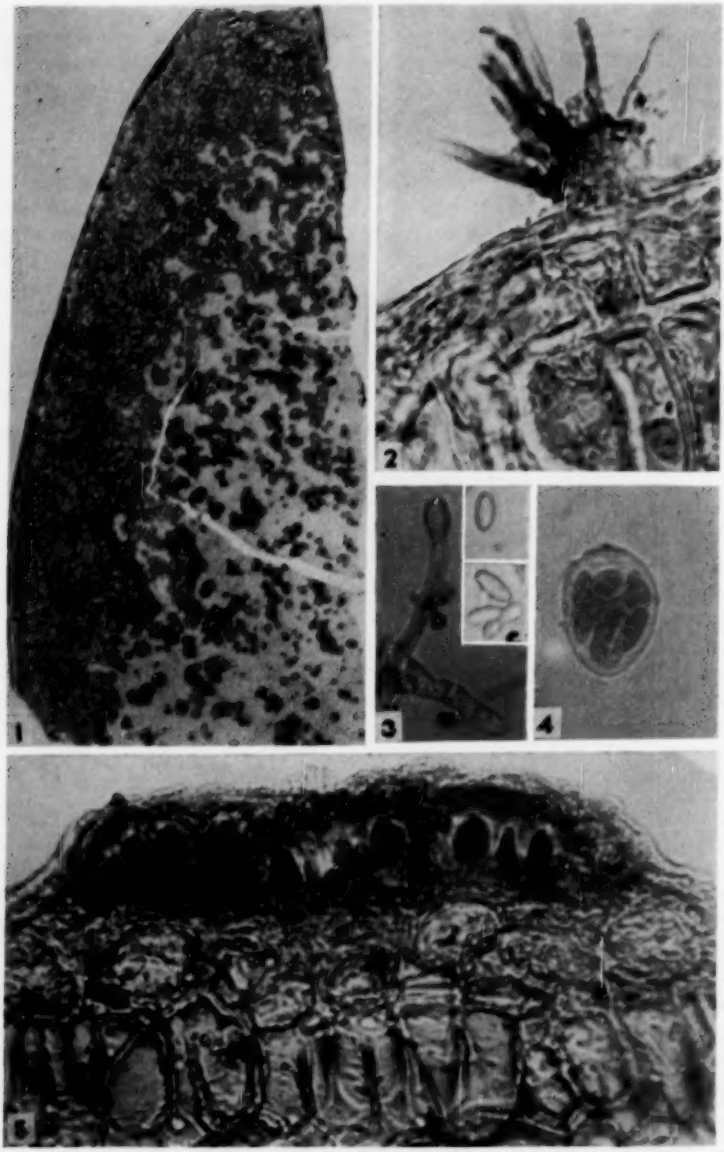
erumpentes, 20–40 μ in diam., 12–24 μ crassi, basi pallida pseudoparenchymatica plus minusve evoluta; conidiophora saepe subuliformia, continua vel basim versus 1-septita; fructificationes acervulares tardius in evotutione probabiliter in sporodochia mutantes.

Hab. in foliis *Magnoliae grandiflorae*, Florida, Georgia, Louisiana, et Mississippi.

Spots epiphyllous, not visible below, isolated and sparse to aggregated and coalescent, often concentrated along the midrib or involving more or less extensive marginal or apical areas of the blade, circular to irregularly angular, slightly elevated, white to gray with a brown margin, up to 1.5 mm in diam.; black, papillose to flattened masses, often solitary at the center of individual spots or scattered over the surface of more extensive discolorations constituting ascogenous or conidiferous stromata; ascocarps subcuticular or intraepidermal in origin, upper surface becoming exposed, subspherical to flattened, 90–240 μ in diam. by 20–40 μ thick, composed of minute hyaline parenchymatous cells beneath and between the asci with a layer of dark thick-walled cells above, forming an epithecium 10–15 μ thick; asci numerous in different stages of development and irregularly dispersed in one or two layers, globose to broadly elliptical, sometimes slightly angular from mutual pressure, sessile, inner wall thickened especially in the apical region, 17.5–30 × 14–20 μ; ascospores irregularly arranged, clavate-ellipsoid, 3-septate with an occasional longitudinal wall, 9–14 × 3.5–6 μ.

Conidial stroma sporodochial or acervular, sporodochium erumpent from a light-colored pseudoparaenchymatous subcuticular stroma externally with dark, thick-walled cells from which the conidiophores arise; conidiophores divergent, straight to geniculate, with as many as 4 septa, dark, sometimes lighter toward the apex, 20–45 × 4–5 μ; conidia acrogenous, borne on short apiculae, 1-celled, ellipsoid, hyaline, 6–12 × 3–5 μ; acervuli intraepidermal-erumpent, 20–40 μ in diam., 12–24 μ thick, light-colored pseudoparenchymatic base scant to well developed, conidiophores often awl-shaped, continuous or 1-septate toward the base; acervular structures with further development probably becoming sporodochial. Sporodochia resemble those of *Sphaceloma perseae* Jenkins (2, Pl. 3).

Distribution: Pathogenic on leaves of southern magnolia (*Magnolia grandiflora* L.; Magnoliaceae), where abundant over large leaf areas, these becoming necrotic, Florida, Georgia, Louisiana, and Mississippi. Type specimen consisting of sporodochia as well as fertile ascomata on year-old living leaves of 1952 gathered shortly before leaf fall from a tree at Athens, Ga., in early June 1953, J. H. Miller. Deposited in the following herbaria: Herb. Dept. Plant Path., University of Ga., Athens, Ga.; Herb. Inst. Biol. São Paulo, Brazil, *6149*, and Mycol. Coll. U. S. Dept. Agri. *91245*, Beltsville, Md., U.S.A.



FIGS. 1–5. *Elsinoë magnoliae* on leaves of southern magnolia, type specimen. 1. Part of an abundantly infected leaf. ×2. 2. Sporodochium with stroma and elongate conidiophores, ×460. 3. Two conidiophores, *a* and *b*, each with an apically borne conidium, *b* also with a conidium borne laterally; *c*, several free-lying conidia, ×460. 4. Ascus with ascospores, ×500. 5. Ascocarp of intra-epidermal origin, showing the irregularly interspersed asci, ×460.

Elsinoë magnoliae was discovered at Augusta, Ga., in 1941 (cf. 3, p. 325), and its definitely known distribution as shown above is still within the host's natural range. This year (1953) on leaves of this tree planted as an ornamental in Rome, Italy, Dr. M. J. Thirumalachar discovered and sent us specimens of a sterile leaf spot that, as he thought, may represent infection by *Elsinoë*. Recent examinations of occasional southern magnolia trees growing in gardens in São Paulo and often as street trees in Curitiba, Brazil, by Dr. Bitancourt and the junior writer, have revealed no evidence of this infection.

The senior writer's observations of infection by *Elsinoë magnoliae* on leaves of the above-mentioned tree began in 1948. That year and each year since as the new growth has appeared it became infected. Stromata developing on the newly formed spots as examined from midsummer on into autumn were immature for asci. Those on the one and two year-old leaves examined early in June 1953 were abundantly fertile. Leaf fall, then not begun, soon ensued and was accomplished for both sets by the end of the month. It is usual for only the second year leaves to fall at this time. Stromata on these leaves continued to be fertile through this period and even for as many as three weeks after leaves had dropped.

Infection of the 1953 leaves, expanding as the 1951 and 1952 leaves dropped, was apparent by July 10. Inoculum available for these new infections would have been ascospores and conidia from their respective fructifications on the old leaves. It is to be expected that other organs of southern magnolia become infected while young, then also harbor the living pathogen. This being the case, they, too, probably also serve as more or less abundant sources of inoculum for infection of new growth of the host.

As seen in the prepared slides, the development of the ascus in *Elsinoë magnoliae* correspond only in part to that previously described by Viégas and Krug (**5**) for *E. mimosae Viégas* (**4**). Ascogenous hyphae such as they reported were not observed in sections of *E. magnoliae*. The ascocarps originate from a hyaline, subcuticular plectenchyma. This develops into a pseudoparenchymatous tissue with dispersed binucleate cells surrounded by minute uninucleate cells. The large fusion nucleus appears later, followed by meiosis. The ascospore cells contain one to several nuclei, probably depending on age. In the dehiscence of the ascus as described for the genus (**1**), the outer inelastic membrane ruptures, permitting the inner elastic membrane to expand and the ascospores to escape through the pore.

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TWO NEW SPECIES OF PHYSODERMA FROM INDIA

B. T. LINGAPPA

(WITH 30 FIGURES)

The present paper concerns two species of *Physoderma* parasitic on higher plants which were found at Banaras, India. One is a very destructive and economically important parasite of jute plants, *Corchorus olitorius* Linn., and the other attacks species of *Commelina, Cyanotis* and *Aneilema*. Both species differ in the characters of rhizomycelium, resting sporangia, zoospores, host reactions and host range from known species of *Physoderma*. Both are therefore regarded as new species and given names.

Physoderma corchori sp. nov.

Rhizomycelio intracellulari effuso, tenui, copiose ramoso, 1.5–2.0 μ diam., ramis rhizoidalibus efficentibus, inflationibus unicellularibus sphericis vel bicellularibus fusoideis intercalaribus praedito. Sporangiis perdurantibus pluribus in cellulas hypertrophicas, globosis et subsphericis, 19–30 μ (media 24 μ) diam., in latere uno depressis, operculo orbiculato, 14–18 μ diam., forma pileo solari simili, in depressione praeditis. Exosporio brunneo, levi, 1.5 μ crasso. Endosporio tenui et hyalino. Contentis luteis oleosis, una vel multis guttulis oleosis praeditis. Sporangiis perdurantibus operculo amoto et endosporio protruso germinatibus. Zoosporis 40 in numero per sporangium, ovalibus, utrinque rotundatis, $3.5 \times 6\,\mu$, antice guttula refractiva distincta praeditis et libere natantibus; flagello 18 μ longo. Zoosporangiis ephemeris epibioticis non visis. Tuberculis conspicuis, hemisphericis vel linearibus, limitatis, gregariis, fulvis, levibus, haud erumpentibus 1–6 × 2–3 × 1–3 mm. magnit., in culmis, ramulis, petiolis, atque nervis *Corchori olitorii* Linn. et *C. acutanguli* Lam. (*C. aestuantis* Blanco). TYPUS: 12 octobris 1952, Banaras, Indiae Or. Leg. B. T. Lingappa.

Rhizomycelium intracellular, profusely branched, tenuous portions 1.5–2.0 μ diam., with numerous fine rhizoids and numerous one- to two-celled intercalary, spherical or spindle-shaped swellings. Resting sporangia numerous in a cell, subspherical or globose, 19–30 μ (av. 24 μ) diam., with a circular depression on one side. Exospore smooth, dark brown, 1.5 μ thick. Endospore thin and hyaline enclosing yellow contents with one or more oily globules. Resting sporangium germinating by protrusion of endosporangium which pushes aside the operculum. Operculum hat-shaped, 14–18 μ diam. Zoospores approximately 40 per

sporangium, oval, 3.5 × 6 μ, with blunt ends, an 18 μ long flagellum and an eccentric refractive globule near the anterior end. Epibiotic phase unknown.

Physoderma commelinae sp. nov.

Rhizomycelio intracellulari effuso, copiose ramoso, crasso, usque 3 μ crasso, ramis tenuiter rhizoidalibus efficentibus, inflationibus sphericis unicellaribus praedito. Sporangiis perdurantibus cellulas implentibus, hemisphericis vel breviter elongatis, lucide brunneis, 24–33 μ (media 30 μ) diam., vel ovalibus 23 × 32 μ magnit., uno latere depressis et operculo in depressione praeditis. Operculo patelliformi et 20–24 μ diam. Exosporio brunneo, levi, 3 μ crasso. Endosporio hyalino. Contentis luteis, una vel multis guttulis oleosis praeditis. Sporangiis perdurantibus operculo amoto et endosporangio protruso germinatibus. Zoosporis 45–50 in numero per sporangium, elongatis, medio angustis, utrinque rotundatis, 5 × 7 μ magnit., flagello 20 μ longo postice praeditis. Zoosporangiis ephemeris epibioticis non visis. Maculis brunneis, limitatis, ovalibus, 3–5 mm. longis, vel elongatis, et 2–3 cm. longis, confuentibus, non erumpentibus. Producit maculas discolores in culmis nec non foliis *Commelinae nudiflorae* Linn., et in culmis, foliis, rachidibus *Cyanotis axillaris* D. Don. et *Aneilemae nudiflorae* R. Br. TYPUS: 30 septembris 1951, Banaras, Indiae Or. Leg. B. T. Lingappa.

Rhizomycelium intracellular, profusely branched, coarse, about 3 μ diam., with numerous rhizoids and intercalary minute, spherical, non-septate swellings. Resting sporangia numerous in a cell, hemispherical, slightly elongate, bright brown, 24–33 μ (av. 30 μ) diam., or oval 23 × 33 μ with a concave side. Operculum convex, 20–24 μ diam. Exospore bright brown, smooth and 3 μ thick. Endospore thin and hyaline, enclosing light yellow contents with one or more oily globules. Resting sporangium germinating by protrusion of endosporangium which pushes off the operculum. Zoospores 45–50 per sporangium, elongate, slightly narrow in the middle, with tapering or blunt ends and a 20 μ long trailing posterior flagellum, and an eccentric refractive globule, 5 × 7 μ. Epibiotic phase unknown.

The type materials will be deposited at the Herb. Crypt. Orient. I.A.R.I. New Delhi; Commonwealth Mycological Institute, Kew, England; U.S.D.A. Bureau of Plant Industry, Beltsville, Md. and New York Botanical Garden, Bronx Park, New York.

Corchorus olitorius Linn., the common jute plant, is extensively cultivated in Bengal and the neighbouring humid regions. It also occurs as a weed, together with its wild relative *C. acutangulus* Lam. Under intensive cultivation the jute plants grow to a height of 12 feet or more branching only at the top, but grow up to 5 feet under wild conditions. Anatomically, jute fibres are phloem in origin and occur in small groups to form radially arranged wedges immediately below an indistinct starch

sheath and abut directly on the later developed phloem without any intervening parenchyma (**5**) (FIG. 16). The xylem elements occur as a compact ring below the cambium and completely encircle the pith. The jute fibre is valued for its strength and durability in the world markets. Production of jute in India was valued at $567 million in 1952 and the area under cultivation is rapidly increasing (**2**).For this reason the discovery of this disease is of immense economic importance.

The disease appears early in August when the plants are frequently submerged in water. By the end of September the stem, branches, petioles and midribs are covered by hemispherical dark brown galls (FIGS. 1, 2). On the leaves the galls occur only on the midribs and veins and never on the lamina. Infection of the veins and pods of *C. acutangulus* results only in local discoloration. Heavy infection is not fatal but may cause stunting. The overcrowded galls on the lower parts of the stem, which remain under water for a considerable length of time, become cracked, undergo decay and appear erumpent and crustaceous. A damping off *Fusarium* sp. was found associated with this region. The galls on all aerial parts remain hard and non-erumpent long after the death of the host plants at the end of the season.

In some preliminary retting experiments, the author noted that the intact strips of fibre strands from infected *C. olitorius* plants could not be extracted. The fibre strands were invariably shattered toward the base and were discontinuous, with numerous holes upward. The fibre strands from properly retted healthy jute stems, on the other hand, are long and continuous, with a more compact basal part and progressively finer distal end (**6**).

For microscopic examination, fresh material bearing sori at different stages of development was fixed in (Allen's) Bouin's solution, cut at 10μ and stained with safranin and light green or with Delafield's haematoxylin. Temporary mounts were also examined in 0.02% lactophenol cotton-blue.

In such materials the tenuous rhizomycelium may be seen to be branched profusely within the host cells (FIGS. 11, 12). The fungus invades all the elements of the stem but is halted at the compact ring of xylem bundles. Xylem and pith cells are therefore never infected. In petioles the xylem bundles lie completely surrounded by the hypertrophied host cells which are filled with the resting sporangia. The rhizomycelium gives out finely branched rhizoids inside the host cells (FIG. 12). It was often possible to trace a long rhizomycelium traversing across several cells before branching. The rhizomycelium bears numerous minute, spherical, intercalary swellings which may be one-

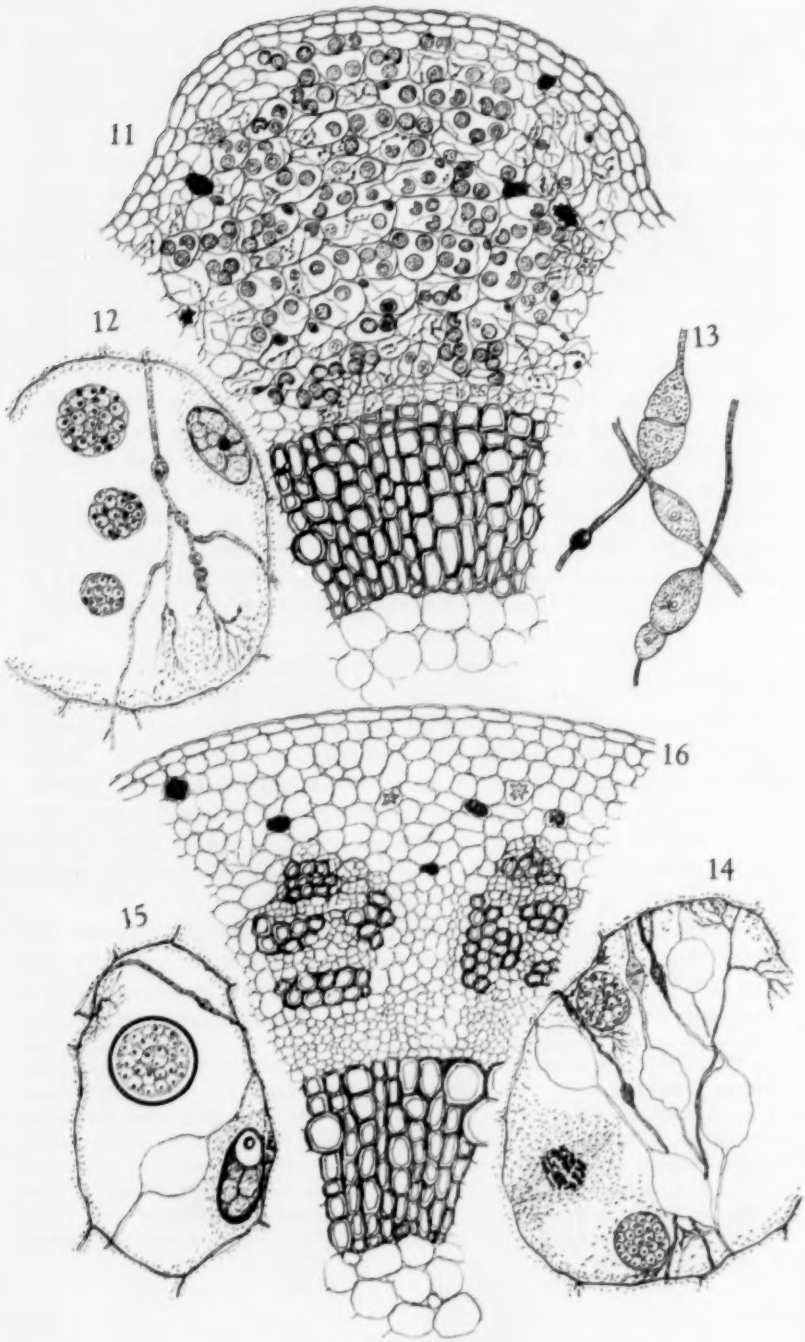
FIGS. 1–10.

celled and spherical or two-celled and fusiform (FIG. 13). These swellings grow bigger and appear empty later (FIG. 14). Numerous observations have led to the conclusion that the delicate protoplasmic strands at the end of the rhizoids have subterminal or intercalary uninucleate swellings which develop into resting sporangial initials. The resting sporangia do not develop either by the transformation of the intercalary swellings on the main branches of the rhizomycelium or as their outgrowths. The young resting sporangia are multinucleate, thin-walled and with deep staining granular contents (FIGS. 12, 14). No haustoria or any appendages were observed on the developing resting sporangia and on the intercalary swellings. As the initials of the resting sporangia are formed, the host cells are greatly enlarged. The host nucleus becomes larger and vacuolate. Isolated cases of the division of the host nucleus in the deeper cells of the cortex were also observed (FIG. 14). However repeated nuclear division has not been observed and any addition of new host cells due to infection is doubtful. The conspicuous galls on the host appear, therefore, to be due to the hypertrophy of the infected cells (FIGS. 11, 16). The infected host cell contents are not degenerated. Infection is not systemic and is confined to an area of a few millimeters. The developmental history is the same in both *C. olitorius* and *C. acutangulus*.

The rhizomycelium invades the phloem elements as well as the fibre bundles. This fungus seems to have some special affinity toward the phloem tissue as is suggested by the longitudinal spread of the rhizomycelium along the midribs and veins and never in the mesophyll. In the stem also the fungus makes more rapid advance towards the phloem. Consequent to infection the fibre cells are greatly enlarged, remain thin walled without attaining any mechanical strength, and the fibre bundles are not differentiated (FIGS. 11, 16). This morbidity affects the retting quality as already noted.

The resting sporangia are formed as the rhizomycelium spreads and at maturity of the sorus masses of resting sporangia are present in the host cells. The resting sporangia are subspherical to globose with a depression on one side in which occurs a hat-shaped operculum (FIG. 9).

FIGS. 1–10. *Physoderma corchori*. 1. A branch of *Corchorus olitorius* Linn. showing symptoms, × ½. 2. A branch of *C. acutangulus* Lam. showing symptoms, × ½. 3. Resting sporangia from mature sorus, × 480. 4. Resting sporangia before germination, × 480. 5. Beginning of germination, the operculum being raised, × 480. 6. Fully formed endosporangia, the types of displacement of operculum, × 480. 7. Germination: zoospores escaping one by one through the apical pore, × 480. 8. Empty sporangial cases, with operculum and endosporangial wall, × 480. 9. Shape of the operculum of *P. corchori*, × 750. 10. Shape of the operculum of *P. commelinae*, × 600.

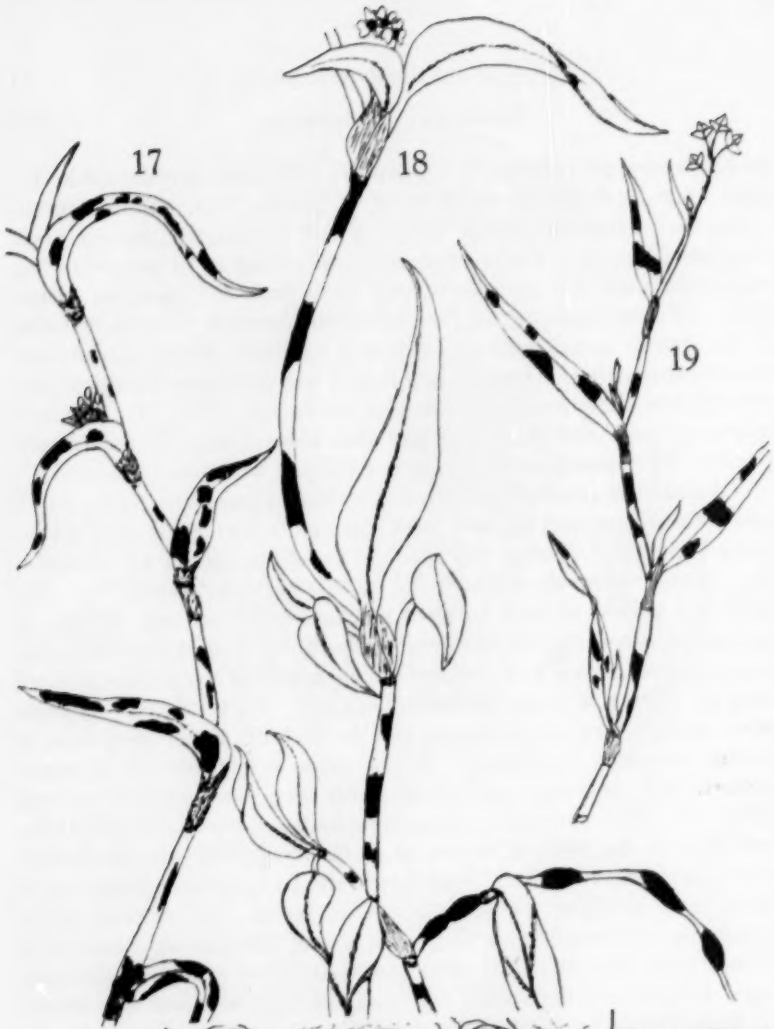


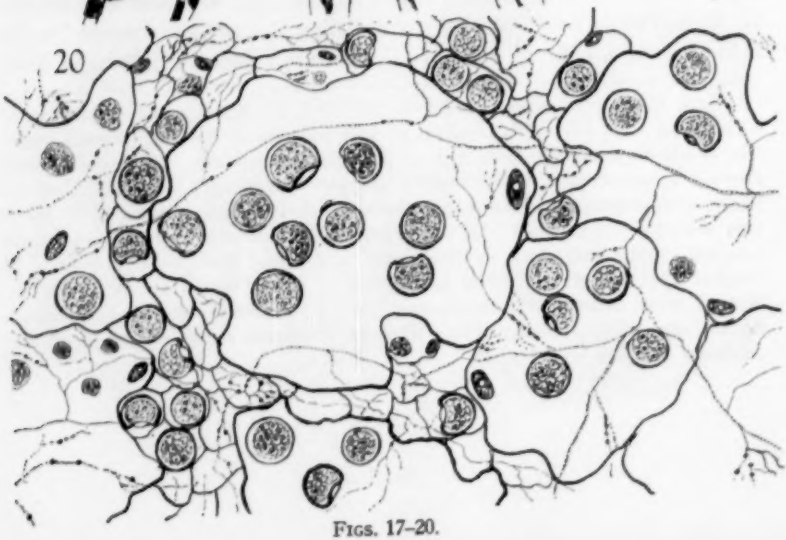
FIGS. 11–16.

Resting sporangia collected in September, 1952, were germinated in distilled water, in 6–12 days at 75–80° F, in March, 1954. During germination the central oily globule breaks up and its materials become finely dispersed (FIG. 4). The operculum is slowly lifted up by the protruding endosporangium and may be carried away from the exospore (FIGS. 5, 6). The endosporangium does not protrude more than the diameter of the resting sporangium in which it is formed. When fully formed the endosporangium is tapered at the apex and develops a subapical pore through which the zoospores come out one by one (FIG. 7). The zoospores are oval with blunt ends and soon assume vigorous movements. Further development of the zoospores has not been observed.

Physoderma commelinae infects *Commelina nudiflora* plants which inhibit the ditches and the road sides abundantly during the rainy season (July–October). During August, light brown to dark, 1 to 1½ inches long patches were observed on the nodes and internodes (FIG. 18). With the advent of cold weather the plants dry up and, having no mechanical tissue, the entire stem shrivels up. The dark patches on the stem, however, retain their original thickness and as a result the affected stem on drying appears beaded (FIG. 18). These "beads" crumble when crushed between the fingers and the dark brown powdery mass of resting sporangia is released. In the same neighbourhood, *Cyanotis axillaris* and *Aneilema nudiflorum* plants were also severely infected (FIGS. 17, 19). *C. axillaris* shows conspicuous symptoms on the leaves and stem in the form of brown to black, irregularly circular patches which may coalesce. On *A. nudiflorum* similar symptoms appear on all aerial parts including the pedicels and the rachis. In hundreds of infected plants observed, not a single case of leaf infection was observed in *C. nudiflora*, whereas on the other two hosts leaves were most conspicuously infected and the beaded aspect of the caulicolus sori was absent. In the sori of the other two hosts, the resting sporangia are not so loosely held as in the sori on *C. nudiflora*. Except for these deviations in char-

FIGS. 11–16. *Physoderma corchori*. 11. T. S. through a sorus in the stem of *C. olitorius*, × 96. Note the absence of differentiation of the fibre bundles, infection of phloem and the hypertrophy of the infected host cells. 12. A cortical cell showing rhizomycelium, rhizoids with intercalary swellings; vacuolated and enlarged host nucleus and young multinucleate resting sporangia, × 500. 13. Types of intercalary swellings, × 930. 14. A cortical cell with developing resting sporangia, enlarged empty intercalary swellings and a dividing host nucleus, × 480. 15. Nuclear condition of the resting sporangium, × 500. The nuclear membrane encloses a large vacuole with conspicuous eccentric nucleolus. 16. T. S. through a healthy stem of *C. olitorius*, × 96. Note the prominent wedge of groups of fibre bundles. Compare with FIG. 11.





FIGS. 17–20.

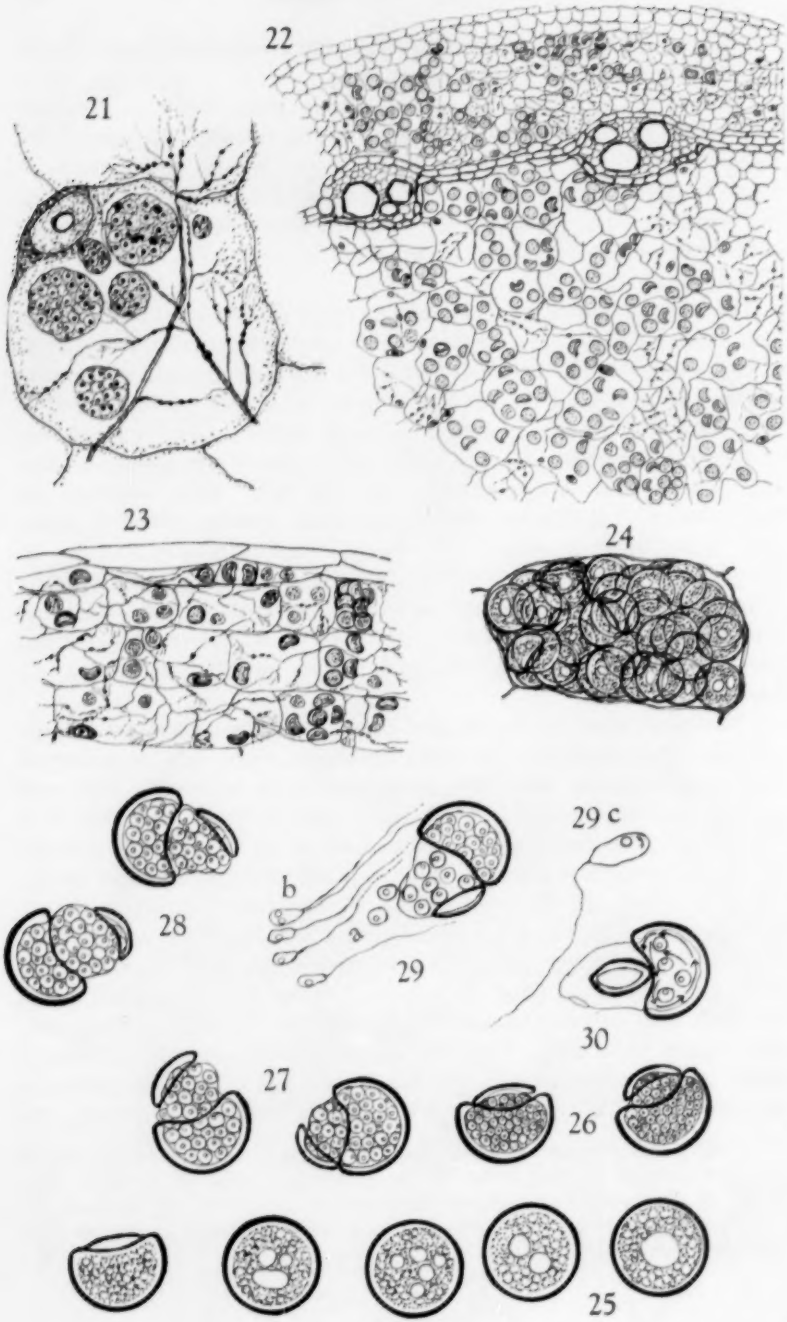
acters on the three commelinaceous hosts the essential symptom is only the discoloration of the affected parts.

Fresh and microtome preparations were made as in *P. corchori*. The rhizomycelium is relatively coarse. The rhizoids, the origin, development, and the nuclear condition of the resting sporangia were found to be essentially similar to *P. corchori* (FIGS. 20–23). The resting sporangia are hemispherical or slightly elongate, each with a concave depression on one side in which occurs a convex lid, the operculum (FIGS. 10, 25).

The rhizomycelium invades the mesophyll and all the tissues of the stem except the vascular bundles, which are symmetrically arranged in a ring of endodermis in *C. nudiflora*. The resting sporangia are numerous and generally fill up the entire lumen of the host cell (FIG. 24). The number of resting sporangia in a cell depends on the natural size of the respective host cell as is evidenced by the entire absence of any enlargement of affected host cells (FIGS. 20, 22). In *C. nudiflora* the large pith cells are naturally encircled by much smaller cells all of which are equally infected without any change of proportionate size due to infection (FIG. 20). The division of the host cells due to infection was never observed, but the host cell nucleus becomes enlarged and vacuolate. The host cells, which are compactly filled up with resting sporangia, resist shrinkage on drying and give rise to the bead-like sori in *C. nudiflora*.

The plant parts bearing the sori of *P. commelinae*, collected in September, 1951, were kept on herbarium sheets. The sori so preserved were wetted several times and germinated easily in March, 1952, and again in February and May, 1954, in a variety of media at 80–85° F in 7–9 hours. The entire process of germination by the protrusion of the endosporangium is essentially similar to the description given for *P. corchori* (FIGS. 25–30). About 45–50 zoospores are produced per sporangium. The zoospores are elongate, narrow in the middle with blunt ends and show typical chytrid movements soon after escaping from the sporangium (FIG. 29 a, b, c). Often a few zoospores fail to escape in time and develop swirling movements within the resting sporangial case (FIG. 30). They too, escape through the pore sooner or later. Giant zoospores with two flagella and two oil globules were also observed (FIG. 29 b). After 5–6 hours of vigorous movement, the

FIGS. 17–20. *Physoderma commelinae*. 17. Symptoms of local discoloration of *Cyanotis axillaris* D. Don, × ½. 18. Symptoms on *Commelina nudiflora* Linn. × ½. 19. Symptoms on *Aneilema nudiflorum* R. Br., × ½. 20. Infected pith region of *C. nudiflora*, × 190. Note the large cells surrounded by smaller cells, both equally infected and showing no hypertrophy.



FIGS. 21–30.

zoospores exhibit slower and halting movements and their anterior ends frequently become tapered, lobed and behave like pseudopodia. The sedentary zoospores also show amoeboid movements for a while. Further development of the zoospores was not observed. The results of some of the germination experiments are given in TABLE I. The results indicate that the resting sporangia germinate better in ordinary water at about pH 8.

TABLE I

EFFECT OF DIFFERENT MEDIA AND *pH* ON THE GERMINATION OF RESTING SPORANGIA OF P. COMMELINAE AT 80–85° F

Medium used	After 16 hours	After 36 hours
Distilled water	Good germination[1]	Many active zoospores
Tap water[2]	Good germination[1]	Many active zoospores
Charcoal water	Good germination[1]	Many active zoospores
Deionized water[3]	Good germination[1]	Many active zoospores
1% Soda Bicarb[4]	Good germination[1]	Many active zoospores
5% Soil extract[4]	Good germination[1]	Many active zoospores
1% Dextrose[4]	Good germination[1]	Many active zoospores and numerous protozoa etc.
1% $(NH_4)_2SO_4$[4]	Poor germination	No zoospores
Pieces of tender host tissue[4]	Poor germination	Many active zoospores
0.1% H_2O_2[4]	No germination	No zoospores and no protozoa etc.
Tap water pH 8	Good germination[1]	Many active zoospores
Distilled water pH 8	Good germination[1]	Many active zoospores
Distilled water pH 7	Good germination[1]	Many active zoospores
Distilled water pH 6	Poor germination	Few zoospores
Distilled water pH 5, 4	No germination	No zoospores
Distilled water pH 9	No germination	No zoospores

[1] Above 20% in different stages of endosporangial formation.
[2] Fresh from deep bore well, pH 8, nonchlorinated.
[3] From Amberlite, Monobed Deionization. Rohm & Haas Co., Philadelphia, Pa.
[4] In distilled water.

FIGS. 21–30. *Physoderma commelinae*. 21. An infected cortical cell of *C. nudiflora*, × 430. Note the rhizomycelium, rhizoids and intercalary swellings, young resting sporangia and enlarged host nucleus. 22. T. S. through a developing sorus in the internode of *C. nudiflora* showing the uninvaded vascular bundles, × 68. 23. L. S. through a sorus in the stem of *C. axillaris* showing the spread of the fungus, × 93. 24. A host cell filled with resting sporangia, teased out from the bead-like sorus in *C. nudiflora*, × 300. Note the retension of the original form of the cell due to the compact mass of resting sporangia inside. 25. Resting sporangia before germination, × 460. Note the dispersal of oily globules. 26. Beginning of germination, the operculum being lifted by the protruding endosporangium, × 460. 27, 28. Fully formed endosporangia, × 460. 29. Escape of zoospores, × 460. a, zoospores escaping slowly one by one. b, occasional giant zoospores. c, enlarged active zoospore, × 900. 30. Swirling movements of a few zoospores within the resting sporangial case before escape, × 460.

The genus *Physoderma* includes species that produce only local discoloration, as in *P. commelinae,* and also those that produce visible hypertrophies on their hosts, as in *P. corchori.* Until very recently, those species that produced hypertrophic symptoms were maintained under the genus *Urophlyctis.* Magnus (**7**) tried to maintain the genus *Urophlyctis* of Schroeter (**8**), which was already losing ground, on the basis that the members of *Urophlyctis* incite hypertrophies of the affected host parts and that their resting sporangia are hemispherical and flattened on one side whereas the species of *Physoderma* produce only local discoloration of the affected parts and that their resting sporangia are globose and ellipsoidal. Known records of the types of germination of the resting sporangia of *Urophlyctis* were by the cracking of the wall, unlike *Physoderma,* in which the germination is known to be by the formation of the endosporangium (cf. Karling, 1950). Karling (**4**), taking into consideration the accumulated evidence, finally merged the genus *Urophlyctis* in *Physoderma.* He considers that the host reactions cannot be of generic significance and that the various special characters of *Urophlyctis* are not always correlated with the host symptoms. As is evident from the foregoing account, *P. corchori* and *P. commelinae* show two distinct types of host reactions. Whereas in *P. commelinae* the resting sporangia are hemispherical to ellipsoid, in *P. corchori* they are subspherical to globose and they both show same type of germination of the resting sporangia. The author also collected *P. aeschynomensis* Thirum. & Whiteh. in its type locality in 1950 and has several times observed the germination of its resting sporangia by the formation of endosporangia. The anomalous method of germination which was described by its authors, in Bangalore material, was never observed. *P. aeschynomensis* also incites marked hypertrophy of the affected parts (**10**). It is thus evident that there is no correlation between the type of host reaction and the shape and germination characteristics of the species of *Physoderma* and *Urophlyctis.* Therefore merging the genus *Urophlyctis* in *Physoderma* appears convincing.

It is further pointed out that *P. corchori* is an addition to the chytrid parasites which are capable of invading the vascular bundles (**1**). It does not attack the xylem bundles. In this connection Thirumalachar and Pavgi (**11**) are not justified in considering *Carpenterella* sp. as a chytrid parasitizing the vascular bundles. Tehon and Harris (**9**) considered their new genus *Carpenterella,* together with *Ligniera vasculorum* (Matz) M. T. Cook, as chytridaceous vascular parasites. While *L. vascularum* is a doubtful member of the *Plasmodiophoraceae,* the systematic position of *Carpenterella* is in serious doubt. Karling (**3**),

with considerable reluctance, treated this genus within the limits of the *Olpidiopsidaceae* in the *Holobiflagellomycetes*. Inclusion of *Carpenterella* within the chytrids will be justifiable only after better knowledge, especially of the zoospore phase of the fungus, is available. As it stands today it has affinities with the parasitic slime molds on the one hand and holocarpic biflagellates on the other. Future investigations of *Physoderma* species, especially of those that incite marked hypertrophy, will doubtless reveal more species capable of parasitizing the vascular elements.

The author is grateful to Prof. J. S. Karling for valuable advice in the present investigation, to his wife for the preparation of the illustrations, to the Curators of the New York Botanical Garden and the Indian Botanical Garden, Calcutta, for the identification of host plants and to Drs. M. D. Whitehead and J. G. Dickson for sending reprints and some herbarium specimens.

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TAXONOMY OF THE SPECIES OF ISOACHLYA POSSESSING SINGLE OOSPORES [1]

T. W. JOHNSON, JR. AND JACQUELINE SURRATT

(WITH 34 FIGURES)

The presence of either glomerulate or clustered oögonia, and the predominance of a single oöspore are characteristics which *Isoachlya intermedia* (Coker and Harvey) Coker (**2**, **4**), *I. unispora* Coker and Couch (**1**), *I. subterranea* Dissmann (**3**), *I. itoana* Nagai (**6**), and *I. glomerata* Richter (**7**), have in common. If the original descriptions and illustrations of these species are compared, other features of similarity are apparent: general configuration of the oögonia, oöspore type, oögonial wall pitting, position of the oögonia on the hyphae, and shape and mode of discharge and renewal of the zoösporangia. Furthermore, there is a rather close intergradation of oögonia and oöspore sizes of at least three of these taxa.

Kelman (**5**) isolated *Isoachlya itoana* from soil in North Carolina, and after a comparison of his isolate with other species, concluded that differences between *I. itoana* and *I. subterranea* were ". . . probably not great enough to consider them separate species" (**5**, p. 209). He thought it unwise, however, to reduce *I. subterranea* to synonymy. The results of a recent comparative study of thirty-two specimens of the single oöspore species isolated from clay soils in Mississippi, indicate that Kelman was correct in his supposition that these two species were the same. Furthermore, our obervations on the living isolates showed that the taxonomy of all of the single oöspore species of *Isoachlya* was, in fact, rather complex.

It is the purpose of this paper to present the results of an investigation into the taxonomy of the five single oöspore species of *Isoachlya*. All living specimens were propagated as single spore isolates on halves of hempseed in sterile, charcoal-filtered, distilled water. Cultures were incubated at 22° C, and were allowed to grow for three weeks before examination.

[1] A portion of the cost of this investigation was defrayed by a Faculty Research Grant from the University of Mississippi and the Board of Trustees of State Institutions of Higher Learning. The authors express their sincere appreciation to Dr. F. K. Sparrow, University of Michigan, for his critical evaluation and editing of the manuscript.

After a study of fourteen of the specimens, it was apparent that they were equally representative of three previously described species: *Isoachlya itoana, I. subterranea,* and *I. glomerata.* The papers describing these fungi (**3, 6, 7**) lead one to the conclusion that none of the three authors knew of or considered the previously described species. The descriptions of *I. itoana* and *I. subterranea,* for instance, appeared in publications issued only one month apart. The illustrations of these two taxa point unmistakably to their being alike. TABLE I summarizes

TABLE I

COMPARISON OF *Isoachlya itoana, I. subterranea,* AND *I. glomerata* WITH FOUR INTERGRADES, IN CHARACTERISTICS OF CRITICAL IMPORTANCE IN IDENTIFICATION

Characteristic	*I. itoana*	Forms				*I. subterranea*	*I. glomerata*
		525	*532*	*560*	*569*		
Oöspore number	1, rarely 2 or 3	1, rarely 2 or 3	1, rarely 2	1, very rarely 2	1, rarely 2	1, extremely rarely 2	1
Oöspore type	Centric, subcentric	Same	Same	Same	Same	Same	?
Oöspore size	31–33μ	35–39μ	32–38μ	38–43μ	38–40μ	39–42μ	23–40μ
Oögonial size	31–48μ	45–54μ	45–50μ	45–55μ	40–50μ	50–55μ	32–51μ
Wall pits	Usually pitted	Usually pitted	Rarely pitted	Unpitted	Rarely pitted	Unpitted	Unpitted
Diclinous antheridia	Present	Lacking	Present	Present	Present	Lacking	?
Monoclinous antheridia		Present in all cases					
Androgynous antheridia		Present in all cases					
Predominant antheridial branch origin		Androgynous in all cases					
Configuration of sex organs		Similar in all cases					
Glomerulate or clustered oögonia		Present in all cases					

the critical identifying characteristics of *I. itoana, I. subterranea,* and *I. glomerata,* together with features of four of the Mississippi isolates.

It is difficult to appraise these data (TABLE I), lacking the opportunity to examine type specimens of the three described species. A comparison of the morphology of oögonia, antheridial branches, zoösporangia, and gemmae, *in toto,* of the living specimens with the descriptions and illustrations of the known species leaves little doubt as to their similarity. While these taxa do exhibit (*sensu strictu*) minor differences, we have isolated variants which possess intermediate features. There are forms which have, for example, smaller or larger oöspores

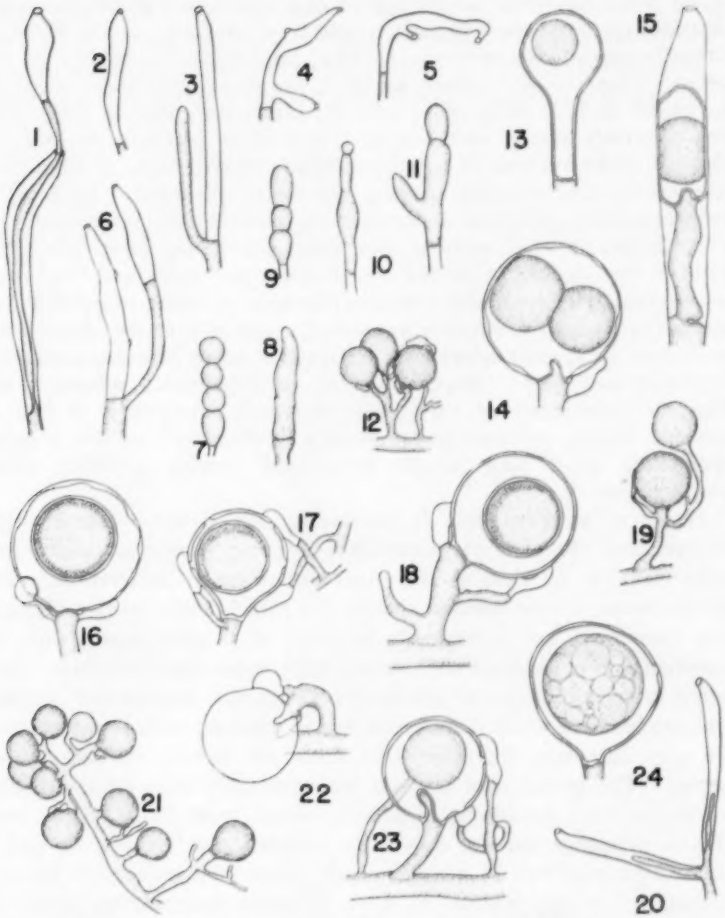
and oögonia than are described for the types, but when a number of variant forms are examined, these size differences represent an intergraded series. Similarly, presence or absence of oögonial wall pitting in the living specimens has been found to be an undependable diagnostic characteristic. The numbers of oögonia with pits, without pits, or with pits only under the point of attachment of the antheridial cells varied considerably. Several hundred oögonia of form *569* were examined before oögonia were found with pitted walls. In other forms, *532* and *701* for example, pits were observed only in oögonia of old, contaminated cultures.

The characteristics of the forms listed in TABLE I are indicative of the features of the remaining ten specimens which were examined. Each variant, should one be inclined to do so, might be raised to varietal or specific rank. If such a procedure were followed, the difficulty in identifying other, and perhaps slightly different forms would be compounded. Since the specimens examined seem to bridge the lacunae between the strictest interpretations of *Isoachlya subterranea*, *I. itoana*, and *I. glomerata*, it is proposed that these taxa be considered synonymous. When a given specimen can be identified equally confidently as any one of three species, it is indicative that the three taxa are too similar to justify their continued recognition as distinct entities.

Isoachlya subterranea, therefore, is retained as the valid entity, inasmuch as it has the prior publication date. The description of *I. subterranea* is emended to include *I. itoana* and *I. glomerata*, and the forms of these which have been isolated. FIGS. 1–34 illustrate the commonly occurring distinguishing features of this species.

ISOACHLYA SUBTERRANEA Dissmann, Beih. Bot. Cent. **58**: 110. 1931.
Isoachlya itoana Nagai, Jour. Fac. Agr. Hokkaido Imp. Univ. **32**: 11. 1931.
Isoachlya glomerata Richter, Flora **131**: 241. 1937.

Mycelium limited, usually dense at periphery of colony; two-week old colony 1–1½ cm in diameter; principal hyphae stout, moderately branched. Gemmae variable in abundance; filiform, spherical, pyriform, or irregular; terminal or intercalary, single or catenulate; functioning as zoösporangia. Zoösporangia abundant in young cultures; fusiform, filiform, frequently bent, curved, or irregular, infrequently branched; variable in length and diameter; renewed internally or sympodially; internally proliferated ones always formed outside the discharged zoösporangium. Zoöspore discharge saprolegnoid, rarely dictyoid; motile spores escaping commonly through a terminal orifice, occasionally through one or more lateral pores. Oögonia abundant; lateral, occa-



FIGS. 1–24. *Isoachlya subterranea*. 1. Secondary zoösporangium showing proliferation outside the primary one, × 70. 2–5. Variations in zoösporangial shape, × 70. 6. Achlyoid type of zoösporangial renewal, × 70. 7–11. Gemmae, × 70. 12. Branched oögonial stalk (glomerulus), × 70. 13. Terminal, unpitted oögonium, × 314. 14. Oögonium with irregular inner wall surface, × 314. 15. Oögonium developed in a discharged zoösporangium, × 314. 16. Terminal oögonium with a centric oöspore, × 314. 17. Pitted oögonium with attendant diclinous antheridial branches, and subcentric oöspore, × 314. 18. Oögonium with a subcentric oöspore, × 314. 19. Branched oögonial stalk. × 70. 20. Branched zoösporangium, × 70. 21. Clustering of oögonia, × 70. 22. Typical bent oögonial stalk and short androgynous antheridial branches, × 314. 23. Lateral oögonium with monoclinous antheridial branches, × 314. 24. Asymmetrical, terminal, pitted oögonium, × 314.

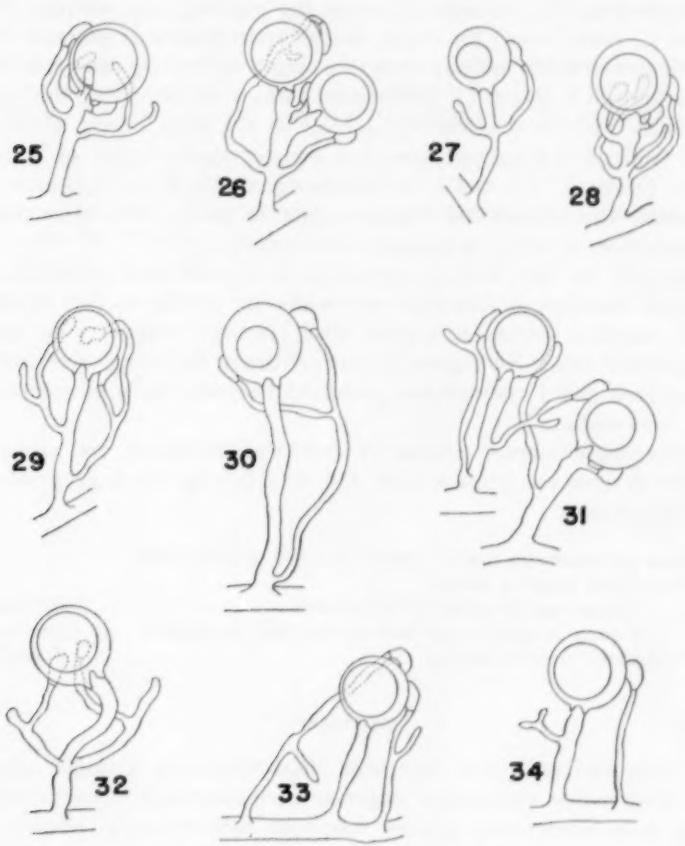
sionally terminal, rarely intercalary or in a discharged zoösporangium; frequently clustered on a hypha; spherical or pyriform, rarely filiform, extremely rarely asymmetrical; 17–58 μ, predominantly 40–50 μ in diameter. Oögonial wall pitted, unpitted, or pitted only under point of attachment of antheridial cells; smooth; rarely irregular on inner surface, extremely rarely with one or a few short papillate projections. Oögonial stalks variable in length; straight, curved, bent, or irregular; occasionally branched, then bearing oögonia in glomeruli. Antheridial branches usually androgynous, occasionally monoclinous, infrequently or rarely diclinous; when androgynous, arising from any point along the length of the oögonial stalk, but occasionally very short, and hidden by the oögonial cell; sparingly branched; lacking on some oögonia. Antheridial cells simple; laterally appressed, extremely rarely attached by projections or apically appressed; fertilization tubes present, persistent. Oöspheres maturing. Oöspores centric or subcentric; spherical, not filling the oögonium; one, rarely two, extremely rarely three or four in number; 14–44μ, predominantly 30–40 μ in diameter; mature oöspore germinating by a long, simple germ tube bearing a small, apical zoösporangium.

Isoachlya unispora and *I. intermedia*, which also possess single oöspores and clustered or glomerulate oögonia, present another taxonomic problem in view of the observations on *I. subterranea*. The characteristics of the former species (of which eight specimens have been examined) are closely akin to those of *I. subterranea*, with the exception that *I. unispora* consistently lacks antheridial branches. Subcentric or centric oöspores,[2] clustered oögonia, and oöspore and oögonial sizes are features which *I. unispora* has in common with *I. subterranea*. We may speculate, therefore, that these are merely variants of one species. The presence of oögonia with attendant antheridial branches in those isolates possessing antheridia varied from 34 to 98 per cent. This is indicative but not conclusive evidence that *I. unispora* and *I. subterranea* may well be a single entity, since they are similar in other respects. For the present, at least, it seems desirable to retain the antheridial-bearing species separate from *I. unispora*, although they are remarkably similar in other morphological features.

A single characteristic of *Isoachyla intermedia* (**2**, **4**) would seem to distinguish it from *I. subterranea*. This is the presence of some irregularly shaped oögonia having wavy or irregular walls which may, in extreme cases, be extended into short papillae. Ten separate isolations of Harvey's species have been studied, comparatively, with the other

[2] Coker and Matthews (**2**) are in error on this point since the figures of *I. unispora* (**1**, pl. 23) clearly show both centric and subcentric oöspores.

single oöspore taxa and their known variants. The characteristic irregular wall of *I. intermedia* is somewhat variable. In three specimens, an average of 68, 73, and 82 percent of the 1000 oögonia examined in each of three subcultures were of the irregular type, the remaining being smooth-



FIGS. 25–34. *Isoachlya subterranea.* Commonly occurring variations in antheridial branch origin, × 95.

walled and spherical. In the other seven specimens, only rarely were oögonia smooth-walled. In addition, the predominant oöspore size of the specimens of *I. intermedia* agreed quite closely with that of the type description (4). That is, no intergrades of any significance in this

criterion were found in *I. intermedia* specimens similar to the intergrades in isolates representing variants of *I. subterranea*.

Kelman (**5**) has pointed out that *Isoachlya itoana* (which we consider to be the same as *I. subterranea*) may be distinguished from *I. intermedia* on the basis of antheridial branch origin. Such branches in *I. intermedia* arise immediately below the oögonial cell, whereas in *I. itoana* (*I. subterranea*) the origin, though androgynous, is generally at a point approximately midway along the length of the oögonial stalk. In the isolates of *I. itoana* (*I. subterranea*) which we examined, this is an extremely variable characteristic. Even in the same colony, androgynous antheridial branches arise in a manner characteristic of both *I. itoana* (FIGS. 27, 32) and *I. intermedia* (FIGS. 18, 22). It is believed, therefore, that antheridial branch origin is not a sufficiently stable characteristic by which to separate these species.

Despite the fact that configuration of oögonia and attendant antheridial branches in *Isoachlya intermedia* are similar to that in other single oöspore species, it appears that Harvey's fungus is an easily recognizable one. No oögonia in any culture of the living specimens of *I. unispora* and *I. subterranea* possessed irregular walls characteristic of *I. intermedia*.

The single oöspore species of *Isoachlya*, therefore, are presently limited to three recognizable taxa, and the following key is proposed for their separation.

Oöspores predominantly single; oögonia clustered or glomerulate
  Antheridial branches present
    Oögonia smooth, spherical or pyriform ..... *I. subterranea*
    Oögonia irregular, outer wall surface wavy or papillate .... *I. intermedia*
  Antheridial branches lacking .....*I. unispora*

SUMMARY

Thirty-two isolates of *Isoachlya*, characterized by a single oöspore and clustered or glomerulate oögonia, were examined. Several specimens were intergrades between *Isoachyla subterranea*, *I. itoana*, and *I. glomerata*. These three are considered to be the same species, for which *I. subterranea* is the valid binomial. *Isoachlya unispora* and *I. intermedia* are discussed. Observations of living specimens indicates that these are valid entities, and easily separable from *I. subterranea*.

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SYNCHYTRIUM RANUNCULI COOK [1]

JOHN S. KARLING

(WITH 31 FIGURES)

In 1947 Cook described *Synchytrium ranunculi* as a parasite of *Ranunculus pusillus* at Baton Rouge, La., and diagnosed it as follows: Galls single and numerous on upper surface of leaves and on petioles, yellowish at first, become reddish and finally black, about 60 μ in diameter; sori varying from pale-yellow to reddish-yellow in color, 12 μ diam.; sporangia 6–8 μ diam. He reported that two parasites may be present in the infected cell and indicated that the initial thallus functions directly as a sorus.

In a study of this species the author found that Cook overlooked some of the critical stages of this parasite and misinterpreted others which he observed. Also, the dimensions of the galls, sori and sporangia which he recorded are inaccurate and too small. Furthermore, what he described and illustrated as two sori in an infected cell are in most instances actually developmental stages of the sorus from the prosorus or initial cell, and this has been confirmed by examination of his own fixed and stained preparations which he kindly sent the author before his death. The presence of a prosorus in the life cycle of a species of *Synchytrium* is very important for its identification and has direct bearing on its ultimate classification in the various subgenera. For these reasons primarily the author is describing the life cycle of *S. ranunculi* with particular emphasis on the development of the prosorus and sorus.

The zoospores of this species are fundamentally similar in structure and behavior to those of other members of the genus. As they emerge from the sporangium they are subspherical to oval in shape (FIG. 1), but when they are actively swimming they become more narrowly oval (FIG. 2) and oblong (FIG. 3). Towards the end of the motile period they become more globular again and almost spherical in shape (FIG. 4). However, after they come to rest the flagellum may lash about, and the zoospore body may occasionally become amoeboid (FIG. 5). The refractive globule is pale-yellow to pale-orange in color and lies near the

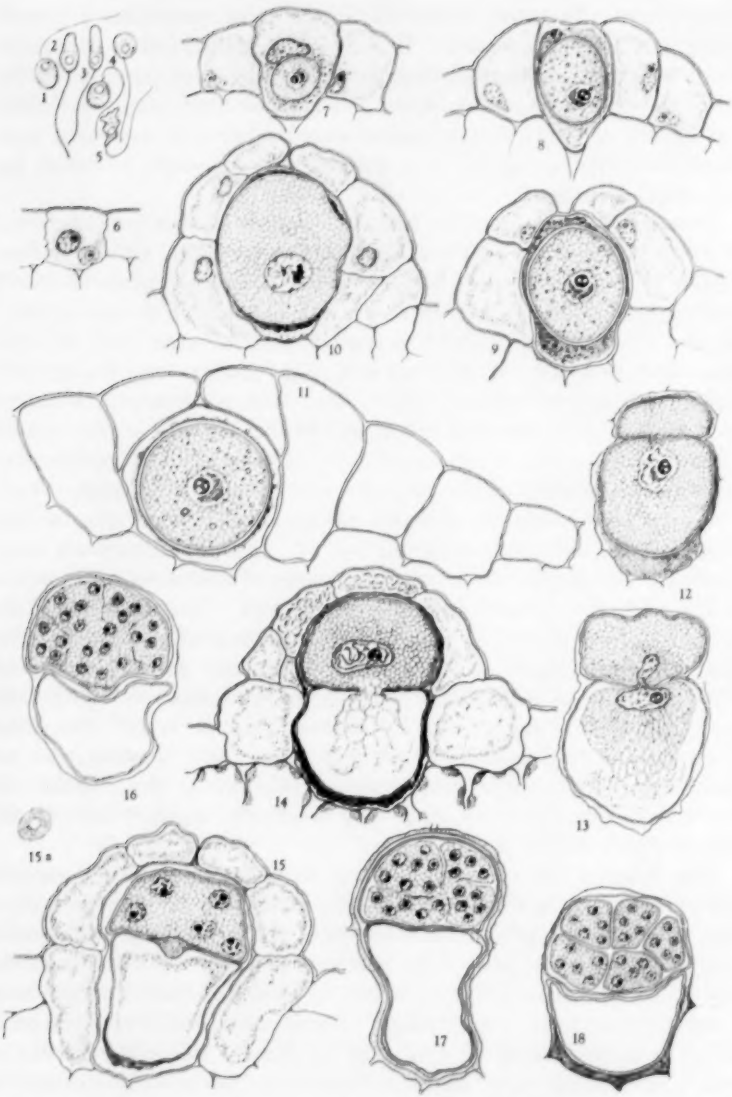
[1] This study has been supported by a grant-in-aid from the National Science Foundation.

posterior end. It varies somewhat in size, and sometimes a second smaller one may be present. Bi-, tri-, and tetraflagellate zoospores occur, and these appear to have arisen by unequal cleavage in the sporangia. At least, no conclusive evidence of their origin by fusion of zoospores or gametes has been observed. Pairs of zoospores were observed swimming together like gametes, but no stages of fusion between them were seen.

Penetration of the zoospore into the host cell has not been observed, but a few young stages of the parasite within epidermal cells have been found. The young parasite frequently lies in the base of the host cell underneath the host nucleus (FIG. 6), and as it grows in size it stimulates the infected epidermal cell to enlarge. At the same time the cytoplasm of the infected cell increases in amount and becomes denser, with numerous suspended granules (FIG. 7). The nucleus also enlarges, but it is not certain whether this is due to the presence of the parasite or the expression of a fundamental ratio between cell and nuclear size. The adjacent healthy epidermal cells also enlarge fairly early (FIGS. 7, 8), and by the time the galls are mature some of them may be four times their normal size as shown in FIG. 11. This figure shows a small, broad and low gall in which the enlargement of epidermal cells extends for a considerable distance from the infected cell. The small cell on the extreme right is normal in size, and a comparison of it with the other cells shows the degree of enlargement which may occur. As the infected cell enlarges in the early stages it grows inward as well as outward, and at this stage (FIG. 7) its base may be deeper than those of the surrounding epidermal cells. In some galls, however, the adjacent cells may elongate inward almost as much as the infected cell. The inward growth of the latter may frequently separate the palisade cells as shown in FIG. 8.

The infected cell appears to enlarge more rapidly than the parasite at first (FIG. 7), so that the latter only partly fills it. Within a short time, however, the growth ratio changes, and as a result the parasite soon fills the greater part of the host cell (FIG. 8). At this stage the host protoplasm is very abundant, and in fixed and stained preparations it often appears to be degenerating. The host nucleus (FIG. 8) appears almost homogeneous in structure and is densely stainable. As far as these observations go it seems to degenerate fairly early, because in slightly older stages it is hardly recognizable.

It is obvious in FIGS. 7–9, and 11 that the presence of the parasite leads to a marked reaction by the host. This consists largely of cell enlargement of the infected and adjacent epidermal cells. No divisions



FIGS. 1–18. *Synchytrium ranunculi.* 1–3. Oval and elongate zoospores, × 695. 4. Zoospores shortly before coming to rest, × 695. 5. Amoeboid zoospores, × 695. 6. Early stage following infection, parasite in base of cell, × 400. 7. Later stage showing enlargement of infected and adjacent epidermal cells, × 337. 8, 9. Still later stages with incipient prosori filling most of host cell and surrounded by dense

of these cells has been observed, so that it is not known whether gall formation in *S. ranunculi* involves meresis also. The position and appearance of the enveloping sheath cells often suggest that they may have divided as well as enlarged, but this remains to be determined from future studies. Nevertheless, the mature sporangial gall (FIGS. 10, 14, 15) is composite and multicellular, and usually approximately half or more of it projects above the surface of the leaf. The latter portion is almost hemispherical in shape and nearly circular in basal outline. The remaining part may be embedded in the leaf tissue and project inward among the palisade or mesophyll cells. Occasionally, the galls may be low and broad (FIG. 11), and project only slightly above the surface of the leaf. Usually, the enveloping sheath is composed of a single layer of enlarged cells, which come together closely at the apex of the gall. Consequently, the pore or opening to the infected cell is relatively narrow. Resting spore galls (FIGS. 21, 22) are fundamentally similar to those which bear sporangia but usually somewhat smaller. Sometimes they may be quite small and project very slightly (FIG. 22) above the surface of the leaf.

As the thallus or incipient prosorus of the parasite develops it becomes invested with 2.4–3 μ thick wall (FIG. 9), as Cook's photographs show quite well. Also, the wall of the host cell becomes thickened (FIGS. 9, 17). As the prosorus reaches maturity, however, its wall appears to be thinner than formerly as shown in FIG. 10. The prosorus shown here is mature and may be taken as representative except for the small amount of residue around it. As shown in Cook's photographs and illustrated in FIGS. 11 and 13, the residue is usually more abundant and may fill the space between the prosorus and host cell wall completely. The prosorus shown in FIG. 10 fills the host cell almost completely and has a large, 17.4 μ diam., nucleus which appears to be in the very early prophase of division. The lunate body (FIGS. 8, 9) which is usually present in resting primary nuclei is absent and appears to be replaced by a number of strands and irregular bodies. Following this stage (FIG. 10) the prosorus develops a broad papilla at the apex, and its

host protoplasm, × 337. 10. Mature prosorus in an almost hemispherical gall, × 420. 11. Almost mature prosorus in a low, broad gall, × 500. 12. Early stage of sorus velopment in apex of infected cell, × 375. 13. Primary nucleus passing into sorus, × 401. 14. Primary nucleus in sorus in prophases of division with 5 or 6 elongate strands, × 285. 15. Early multinucleate sorus with a plug of densely stained material filling opening between prosorus and sorus, × 385. 15A. Plug in cross section, × 400. 16. Multinucleate sori with progressive cleavage furrows, × 317. 17. Sorus with sporangi; basal portion of infected cell constricted by expansion of sheath cells, × 317. 18. Mature sorus with polyhedral sporangia.

protoplasm begins to flow out to form the sorus in the upper part of the host cell. FIG. 12 shows an early stage in this process in which the primary nucleus is approaching the aperture between the prosorus and incipient sorus. This nucleus is slightly beaked at its upper surface, but usually the nucleus at this stage is somewhat flattened or elongated transversely to the long axis of the prosorus. FIG. 13 shows the primary nucleus passing into the incipient sorus, and in FIG. 14 the passage has been completed. As far as these observations go, this passage appears to take place while the primary nucleus is in the prophases of mitosis, and these observations agree fundamentally with those of Miss Curtis (**1921**) on *S. endobioticum*. The nucleus in FIG. 13 includes 5 or 6 strands which may be elongate chromosomes. However, the exact number of chromosomes in *S. ranunculi* has not been determined inasmuch as no late prophases or equatorial plate stages were observed.

It may be noted here that Cook's FIG. 4A illustrates a fairly early stage in the formation of the sorus from the prosorus. The author has examined the slide from which this photograph was taken, and sections adjacent to the one in his FIG. 4A showed the presence of a pore and passage of the protoplasm through it. His FIG. 4B shows the empty prosorus under the sorus, and in FIG. 4D part of the collapsed sorus lies in the base of the host cell. As noted earlier, Cook misinterpreted FIG. 4A and 4B as two parasites in an infected cell.

The migration of the protoplasm into the enlarging sorus is almost complete in FIG. 14, and eventually the prosorus usually becomes completely empty. Occasionally, however, a small amount of residue may remain in it as shown in FIG. 15. Following this stage a conspicuous plug of densely stainable material develops in the opening between the prosorus and sorus (FIG. 15). The origin and development of this plug is not clearly evident in *S. ranunculi*, but in *S. succisae* Rytz (**1907**) believed that it is formed from the residual protoplasm in the prosorus. In cross sections of stages similar to the one in FIG. 15 it appears as an almost circular, densely stained area with a hole in the center (FIG. 15A). The latter apparently closes up completely later. The presence of such plugs is clearly visible in several of Cook's slides of *S. ranunculi*. Such plugs were first described by Rytz (**1907**) in *Synchytrium succisae*, and later Kusano (**1930**) found them in the germinating resting spores of *S. fulgens*. The author has found them in *S. stellariae*, *S. cerastii*, *S. australe*, *S. lindquistii*, *S. achyroclines*, *S. brownii* and another species of *Synchytrium* from Argentina (**1955**) on *Sphaeralcea bonaerensis*. It is quite likely that they will be found in all species in which the initial thallus or resting spore functions as a prosorus.

After the prosorus is emptied the sheath cells around the lower part of the host cell seem to expand, and as a result this part of the infected cell frequently appears slightly invaginated in fixed and stained preparations as shown in FIG. 17. This expansion and invagination may be the result of loss of turgor in the host cell as the prosorus empties. At this stage and later the infected cell has a characteristic appearance in most galls. It appears to be bilocular with the empty prosorus in the lower and the sorus in the upper half.

Following the development of the sorus the primary nucleus completes its division, and subsequently its daughter nuclei divide until the sorus becomes multinucleate (FIG. 16). Progressive cleavage then occurs (FIGS. 16, 17), delimiting multinucleate segments which develop sporangia and become invested with a thin wall (FIGS. 18, 19). The sorus and sporangia then appear to increase in volume and thereby push down into the area occupied by the empty prosorus (FIGS. 18, 19). Apparently as the sorus ruptures the empty prosorus begins to invaginate, and this may continue until it appears as a collapsed vesicle in the base of the infected cell as is shown in Cook's FIG. 4D. The sporangia may then occupy most of the host cell. Later, as the gall dehisces the sporangia are liberated, and if sufficient moisture is present their protoplasm undergoes cleavage and forms zoospores (FIG. 20).

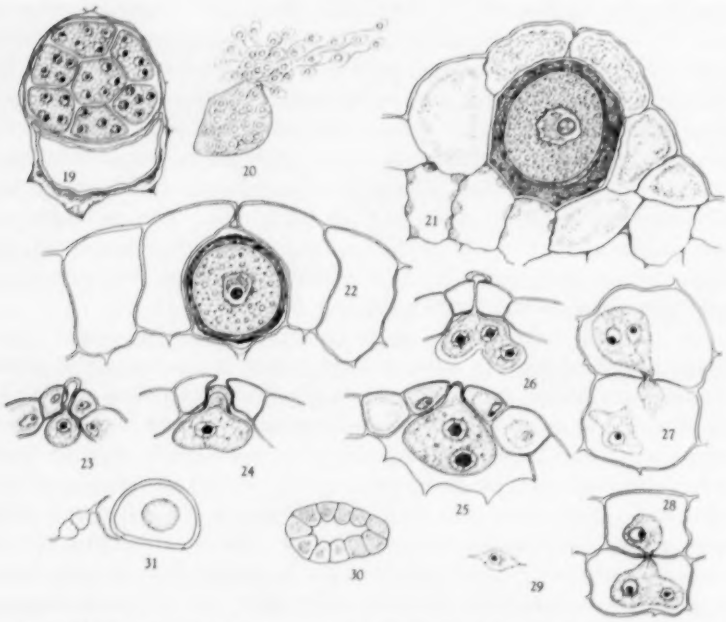
So far only a few resting spore galls and spores have been found. From these it appears as if their development is fundamentally similar to that of the sporangial galls and prosorus. As the author (**1954**) has described for *S. australe*, the incipient resting spores are recognizable by the presence of a large number of globules and densely-staining bodies in the cytoplasm, and as the spores mature they become enveloped by a thick wall. Frequently, the wall is enveloped by a thicker and denser layer of host protoplasmic residue (FIG. 21) than the prosorus, but this is not always true. As shown in FIG. 21 the residue may be very limited in quantity. Germination of the resting spore has not been observed, and it is not known whether it functions as a sporangium, sorus or prosorus.

From the above observations it is now possible to describe *S. ranunculi* more fully and amend the brief diagnosis given by Cook in 1947, as follows:

Prosorus usually solitary, oval, 52–60 × 64–70 μ, subspherical, 54–80 μ with a 2.4–3 μ thick wall; empty prosorus lying in base of infected cell. Sorus subspherical, 48–78 μ, oval, 48–74 × 66–90 μ, or almost hemispherical. Sporangia 12–18 in number, predominantly polyhedral, 18–38 μ in greatest diam., exceptionally large ones up to 48 μ diam.,

with yellowish-orange content and a thin hyaline wall. Zoospores predominantly oval, 2.8–3 × 3–3.8 μ, with a pale yellowish refractive globule. Resting spores oval, 32–38 × 40–48 μ, or almost spherical, 46–52 μ, with coarsely granular content and a 3.8–4.5 μ thick brown wall; germination unknown.

Sporangial galls usually single and scattered or aggregated on upper surface of leaves and on petioles, sometimes confluent, composite, multicellular, oval 96–132 × 120–144 μ, subspherical, 96–126 μ, in median longitudinal section; protruding portion on leaf almost hemispherical,



FIGS. 19–22. *Synchytrium ranunculi*. 19. Expanded sorus invaginating empty prosorus, × 337. 20. Dehiscing sporangium, × 466. 21. Large resting spore gall; resting spore surrounded by a thick, dense layer of residue, × 377. 22. Small low and broad resting spore gall, × 331.

FIGS. 23–26. Unknown organism in substomatal cavities. 23. Uninucleate stages, × 400. 25, 26. Bi- and trinucleate stages, × 441.

FIGS. 27–30. Plasmodiophoraceous organism. 27. Plasmodium-like thallus migrating through walls of deep-lying cells, × 55. 28. Thallus migrating from epidermal to mesophyll cell, × 555. 29. Meront-like uninucleate body, × 380. 30. Cystosorus-like body, × 700.

FIG. 31. Resting sporangium and portion of rhizomycelium of *Physoderma* sp., × 438.

basal portion embedded in host tissue; yellowish at first, then red and finally becoming almost black in color. Resting spore galls fundamentally similar but usually somewhat smaller, 68–74 ×80–98 μ diam.

On *Ranunculus pusillus,* Baton Rouge, La., causing crinkling of leaves.

It is to be noted in the above diagnosis that the dimensions of the galls, sori and sporangia are considerably larger than reported by Cook who listed them as 60 μ, 12 μ, and 6–8 μ diam., respectively.

On the basis of the presence of a prosorus in its life cycle, *S. ranunculi* may be assigned provisionally to the subgenus *Mesochytrium.* However, its ultimate classification depends additionally on the manner of resting spore germination, according to the author's (**1953**) classification of the subgenera. *Mesochytrium* and *Microsynchytrium* are the only two known subgenera whose members develop prosori, and should the resting spore of *S. ranunculi* function as a sporangium in germination it would belong in *Mesochytrium.* On the other hand if it functions as a prosorus *S. ranunculi* should be included in *Microsynchytrium.* Regardless of its final classification, it appears to be a valid species of *Synchytrium* and differs from other known species which occur on *Ranunculus* by the presence of a prosorus. So far five species have been reported on *Ranunculus: Synchytrium aureum* on *R. acer* and *R. montanus; S. andinum* on *Ranunculus* sp. and *R. californicum; S. anomalum* on *R. ficaria; S. cinnamomeum* on *R. recurvatus* and *R. septentrionales,* and *S. ranunculi* on *R. pusillus.* Of these *S. aureum, S. anomalum* and *S. cinnamomeum* are reported to be short-cycled species which form only resting spores and belong in the subgenus *Pycnochytrium. Synchytrium andinum* is the only other long-cycle species besides *S. ranunculi* which occurs on *Ranunculus,* but it does not develop a prosorus and is therefore included in the subgenus *Eusynchytrium.*

In his description of *S. ranunculi* Cook reported that its thallus develops to some extent in the substomatal cavities of its host, apparently following entry of the zoospores through the stomata. His Fig. 3G shows what he believed to be an early stage of such a thallus. It is quite possible that such infections occur in *S. ranunculi,* inasmuch as similar ones have been reported by Kusano (**1909**) and Tobler (**1912**) for other species of *Synchytrium.* However, good evidence has been found in Cook's slides that the thalli which he found in the substomatal cavities may not relate to *S. ranunculi* at all. At least two and possibly three organisms in addition to *S. ranunculi* have been found in his sections of *Ranunculus pusillus* leaves and petioles. One seems to relate to a species of the Plasmodiophoraceae, and another one is a species of *Physoderma.*

Early stages in the development of an organism similar to the one shown in Cook's Fig. 3G are fairly abundant in his slides. FIGS. 23 and 24 show uninucleate stages of this organism while FIGS. 25 and 26 show bi- and trinucleate stages, respectively. In each stage the thallus in the substomatal cavities is connected by a fairly long (FIGS. 23, 26) or short (FIGS. 24, 25) tube or neck to a thick-walled empty cyst which may lie outside or between the guard cells. The latter may be closed (FIG. 26) or open (FIGS. 23–25), and the cysts look like empty zoospore cases whose walls have become thickened on one surface. The nuclei of these thalli are not very similar to the primary nucleus of *Synchytrium* species and often show a "wheel-like" internal structure which has been reported as characteristic of several species of the Plasmodiophoraceae (Karling, **1942**). Unfortunately, Cook did not use No. 0 coverglasses on his slides, and it is impossible to study these nuclei under the oil immersion lens without smashing the preparations.

That these thalli do not relate to *S. ranunculi* seems evident from their bi- and multinucleate character. As noted above, the primary nucleus of *S. ranunculi* does not divide until it has migrated into the incipient sorus, and a multinucleate stage does not appear until very late in the development of *Synchytrium* species. Here, however, the thalli are bi- and multinucleate in the early stages. These thalli are enveloped by a distinct wall which becomes quite evident in plasmolized specimens (FIG. 26). No additional developmental stages of these organisms have been found, and it is not certain that they relate to the plasmodiophoraceous parasite to be described next. It is not improbable that they might be developmental stages of an endophytic alga or possibly a species of the family Olpidiaceae.

The plasmodiophoraceous parasite occurs in most cells of the leaf and petiole sections of *Ranunculus pusillus* and is plasmodium-like in shape and structure. FIG. 27 shows a portion of a plasmodium passing through the wall of adjacent deep-lying cells of the petiole, and in FIG. 28 is shown a stage of migration from a leaf epidermal cell. A few spindle-like uninucleate bodies (FIG. 20), which look very much like meronts, were observed in several host cells. In addition to these bodies and plasmodia numerous cytosori-like multicellular structures were found. These were irregular, oval, oblong and almost spherical in shape and consisted of a few to numerous cells or cysts (FIG. 30). Also, several stages of their development were present, and from these stages it appears that they were derived from plasmodia such as shown in FIGS. 27 and 28.

The third parasite found in Cook's slides is a species of *Physoderma*.

Numerous resting sporangia, 18–22 × 30–38 μ, and remnants of a rhizomycelium (FIG. 31) were present in the deep-lying cells of the petiole.

SUMMARY

Synchytrium ranunculi develops a distinct prosorus in its life cycle, which gives rise to a sorus in the apical part of the infected cell. On this basis it may be included provisionally in the subgenus *Mesochytrium*. In addition to this parasite, Cook's slides of *Ranunculus pusillus* leaves and petioles include three other organisms; a plasmodiophoraceous parasite, a species of *Physoderma*, and possibly an endophytic alga.

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AN INDEX TO L. O. OVERHOLTS' MYCOLOGICAL NOTES

CHARLES L. FERGUS [1]

Dr. L. O. Overholts published a series of thirteen papers entitled "Mycological Notes." They contained descriptions and photographs of new and unusual fungi. All but one (Mycological Notes for 1920. Bull. Torrey Bot. Club **49**: 163–173. 1922) were published in volumes **12–34** of MYCOLOGIA (1920–1943). The following index of the genera and species described in these articles is presented here with the hope of aiding mycologists in using them. The volume, pages, and date cited refer in each case to MYCOLOGIA except for the 1922 references, in which case these refer to the Bulletin of the Torrey Botanical Club.

abietina (*Pistillaria*) **26**: 512–513. 1934.
abietinum (*Toxosporium*) **26**: 505–506. 1934.
abietis (*Valsa*) **21**: 278–279. 1929.
acerina (*Stilbella*) **35**: 253. 1943.
acerinum (*Sphaeronema*) **17**: 110. 1925.
acerinum (*Steganosporium*) **26**: 505. 1934.
acerinus (*Aleurodiscus*) **25**: 426. 1933.
admirabilis (*Polyporus*) **22**: 244. 1930.
agglutinans (*Hymenochaete*) **16**: 235. 1924.
agoseridis (*Ramularia*) **30**: 272. 1938.
albobrunnea (*Poria*) **32**: 262–263. 1940.
alborosella (*Isariopsis*) **30**: 271. 1938.
albostramineum (*Corticium, Gloeocystidium*) **30**: 275–276. 1938.
allii (*Heterosporium*) **26**: 503. 1934.
alnea (*Tympanis*) **22**: 237. 1930.
alni (*Naemospora*) **35**: 252. 1943.
ambigua (*Poria*) **17**: 108–109. 1925.
americana (*Wynnea*) **16**: 236. 1924.
americanum (*Pucciniastrum*) **26**: 508. 1934.
amorphus (*Aleurodiscus*) **25**: 426. 1933; **26**: 508. 1934.
andropogonis (*Uromyces*) **35**: 247–248. 1943.
angulosis (*Cryptodiscus*) **25**: 419. 1933.
angustata (*Coronophora*) **21**: 275–276. 1929.
apiculatum (*Corticium*) **21**: 280–281. 1929.
apiculatus (*Aleurodiscus*) **26**: 508. 1934.
aquatica (*Lemonniera*) **30**: 271. 1938.
arnicole (*Entyloma*) **26**: 507. 1934.
artocreas (*Discosia*) **18**: 180. 1926.
asterophora (*Nyctalis*) **25**: 427. 1933.
atrum (*Arthrobotryum*) **35**: 250. 1943.
aurea (*Ocellaria*) **26**: 506. 1934.
aureum (*Synchytrium*) **35**: 243. 1943.
aureus (*Merulius*) **12**: 138. 1920.

[1] Contribution No. 188 from the Department of Botany, Pennsylvania Agricultural Experiment Station. Authorized for publication June 22, 1954, as paper No. *1882* in the Journal Series.

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NOTES AND BRIEF ARTICLES

Hymenomycetous species forming mycorrhizae with Pinus virginiana

Since Melin [1, 2] first developed methods for the positive identification of ectotrophic mycorrhizal fungi using aseptic culture techniques, it has become standard procedure that before any fungus species may be considered a definite mycorrhizal partner proof be established through aseptic synthetic culture. Positive identities are invaluable to those persons engaged in physiological investigations of mycorrhizae. Consequently, the authors have experimented with several isolates of American and Swedish Hymenomycetes together with *Pinus virginiana* Mill. so that cultures of known mycorrhizal fungi would be available for their studies of this naturally occurring phenomenon.

Pinus virginiana seedlings were grown for one month, using the culture methods described by Hacskaylo,[3] in which the substrate was Terra-Lite moistened with a suitable nutrient solution without glucose. The cultures were then inoculated with mycelial suspensions of fungi in a glucose solution so that the nutrient solution in the flasks contained a concentration of 0.25% or 0.5% glucose. The roots of the seedlings were examined from four to six weeks later. The fungi selected and the results of the tests were as follows: All isolates of the American hymenomycetes, *Amanita caesaria* Schw., *A. frostiana* Peck, *A. rubescens* S. F. Gray (depauperate form), and *Boletus bicolor* Peck, were found to have caused ectotrophic mycorrhizae of the unbranched, singly forked, and coralloid types (Fig. 1). The Swedish isolates already known to be mycorrhizal on *Pinus silvestris* L. in Sweden, kindly provided by Professor Elias Melin at Uppsala, were *Boletus luteus* Fries, *B. variegatus* Fries, *Cenococcum graniforme* Ferd. and Winge, and *Rhizopogon roseolus* (Corda) Th. Fr. Of these, *Boletus variegatus* and *Rhizopogon roseolus* were the only ones which entered into the mycorrhizal relationship. The authors, however, have noted that in the Washington, D. C., area,

[1] Melin, E. Über die Mykorrhizenpilze von *Pinus silvestris* L. und *Picea abies* (L.) Karst. Svensk. Bot. Tidskr. **15**: 192–203. 1921.

[2] Melin, E. *Boletus*-Arten als Mykorrhizenpilze der Waldbäume. Ber. Deutsch, Bot. Ges. **40**: 94–97. 1922.

[3] Hacskaylo, E. Pure culture syntheses of pine mycorrhizae in Terra-Lite. Mycologia **45**: 971–975. 1953.

naturally occurring black mycorrhizal structures, which closely resemble the *Cenococcum graniforme* and *Pinus silvestris* formations, appear on the roots of *P. virginiana* and several other ectotrophic mycorrhizal forest tree species. To date neither isolations nor pure culture studies have been attempted with this fungus.

Of the above isolates, Palmer [4] has found that at least one, *Amanita rubescens,* will form mycorrhizae with the short roots of *Pinus virginiana* which are produced when certain concentrations of beta-indoleacetic acid are introduced into the cultures containing the Terra-Lite substrate.

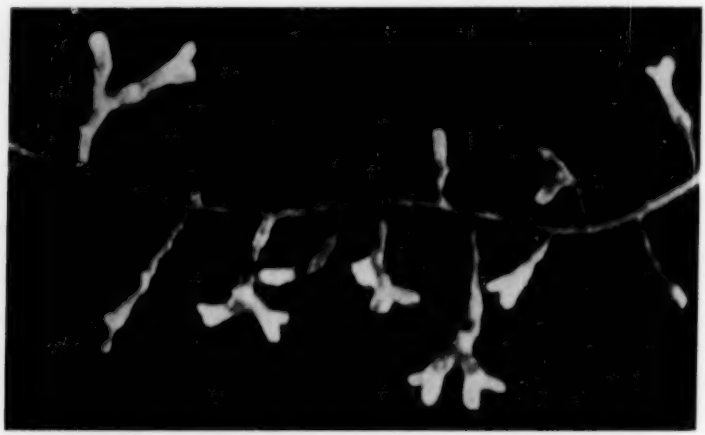

FIG. 1. Ectotrophic mycorrhizae comprising *Pinus virginiana* and *Amanita rubescens.*

Consequently it appears that isolates of hymenomycetes together with the indole-acetic acid could be of great value in increasing the absorbing surface of pine or perhaps other species of tree seedlings prior to transplanting them from nursery beds to plantations.

The possibility of interchanging mycorrhizal fungal species in various parts of the world may also have important implications. It is self-evident that species of fungi can be different physiologically, as is shown by the specificity in mycorrhizal relationships. If one fungus were found to be more efficient than another in the transfer of nutrients from the soil to the tree, its adaptation to other geographical regions as a

[4] Palmer, John G. Mycorrhizal development in *Pinus virginiana* as influenced by a growth regulator. Thesis submitted for the degree of Doctor of Philosophy, The George Washington University. 1954.

mycorrhizal partner on similar or other species might be of practical value.

The senior author wishes to thank Professor Elias Melin of the Institute of Physiological Botany, Uppsala, for providing facilities for the portion of this work done in Sweden.—EDWARD HACSKAYLO[5] AND JOHN GILBERT PALMER, Department of Botany, The George Washington University, Washington, D. C.

THE NOMENCLATURE OF ARMILLARIA, HYPHOLOMA AND ENTOLOMA

In a recent article by Dennis, Wakefield and Bisby (Trans. Brit. Myc. Soc. **37**: 33. 1954), it is proposed to adopt as lectotypes for *Armillaria: A. mellea;* for *Hypholoma: A. lateritius,* and as valid generic name for *Rhodophyllus* Quél., *Entoloma* emend. Donk, Dennis. The three authors have apparently overlooked my paper "Die Nomenklatur der Hoeheren Pilze" (Schweiz. Zeitschr. f. Pilzk. **29**: 204. 1951) where I have shown that (1) the wording used by Kummer leads to the acceptance of new genera like *Armillaria* Kummer rather than to the interpretation of Kummer's genera as new status (*Armillaria* (Fr.) Kummer); (2) that for *Armillaria* Kummer the first selection was mine: *A. straminea* (l.c. p. 228), and that *Hypholoma* Kummer must be considered as a *nomen dubium* since it is impossible to interpret (p. 209); (3) that *Rhodophyllus* Quél. is definitely not homonymous with *Rhodophyllis* Kützig according to the existing rules; and (4) that *Acurtis* cannot be disposed of as the name of a form genus of the Fungi Imperfecti; (5) that the number of species already transferred to *Rhodophyllus* is much larger than the number of species to be transferred to *Entoloma* or, even more so, to *Acurtis* (p. 224).

This leaves for discussion only a few additional arguments such as the identity of *Armillaria* and *Amanita,* which is indicated as a threatening possibility that might lead to the eventual loss of *Armillaria* as a valid generic name. In the first place, Locquin is quite isolated among mycologists who believe that the structural differences between *Armillaria luteovirens* and the Amanitas are too large and significant to combine any *Armillaria* with any *Amanita,* but not even Locquin comes to the conclusion of such a synonymy since he merely synonymizes *Armillaria* and *Aspidella.* Invoking rule 15 against Locquin, the three

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British authors seem to imply that Locquin's work is devoid of "serious motives," which is more than the writer would want to state. But even if *Armillaria* eventually had to be abandoned, there is no reason for regret. I certainly sympathize with the underlying (somewhat nostalgic) tendency of many mycologists, to preserve as many of the Friesian names as possible, and this was one of the reasons why Singer & Smith proposed *Armillaria luteovirens* as lectotype. On the other hand, it is Fries's own fallacious taxonomy which, at times, makes it impossible to preserve his names efficiently, as is the case in *Armillaria*, a notoriously artificial, badly defined, incongruous genus proposed by Fries in spite of the fact that older taxa for the same group were at his disposal. The same is true for *Hypholoma*. It was Karsten's merit to remedy this situation partly, and by doing so, he implied the choice of a lectotype outside the split group proposed by him (*Armillariella*, resp. *Naematoloma*).

The phytopathologist who wishes *Armillaria* maintained for *Armillariella mellea* has, as a review of phytopathological literature during the "reign" of Fries and Saccardo proves, mostly contented himself with the binomial *Agaricus melleus*, later using *Armillaria* and *Clitocybe* (20th century), frequently falling back on *Rhizomorpha*. *Armillariella* is still rather rarely used. This proves merely that there is a natural tardiness on the part of the phytopathologist to adjust himself to the progress of taxonomy aside from an understandable wish to avoid name changes in general. With all due consideration for this wish, often in contradiction with that of the medical mycologist (*Oidium!*), or the mushroom amateur, it is not always easy to find a solution satisfactory to most, without hurting taxonomy.

Dennis *et al* end up by proposing to "treat *Acurtis* as a nomen rejiciendum," apparently in anticipation of the acceptance of a corresponding rule which does not exist and has been rejected in Stockholm as well as in Paris. The fact that it could have been proposed in the case of *Acurtis*, in spite of this, shows with all desirable clarity what would be in store for botanical nomenclature if such a rule ever became a valid article. Instead of remaining restricted to plants of extraordinary economic importance and being proposed only in exceptional cases (as promised), this article would be invoked and committees be swamped by proposals for rejection of taxa wherever there is a desire to avoid the application of the rules. It seems to me that, in the case of *Acurtis*, a rejection of *Rhodophyllus* as nom. conserv. means that the majority of the Committee on Fungi consider it better to apply the nomenclatorial

rules as they stand, i.e. in favor of *Acurtis*.[1]—(ROLF SINGER, Instituto Miguel Lillo, Tucumán, Argentina.

REFRIGERATOR STORAGE PROLONGS AECIOSPORE COLOR AND VIABILITY

Fresh, mature aecia of the various species of rust fungi in the genera *Cronartium* and *Peridermium* are of striking appearance because of the yellow or orange color of the aeciospore masses. When specimens are collected and brought into the laboratory the spores usually fade and become whitish in color within a few months. The specimens thus depreciate rapidly in value for display and instructional purposes.

The natural color of the aecial material may be prolonged for two years or more and viability of the spores greatly lengthened by placing the specimens in paper bags or wrappers and storing them in a refrigerator at temperatures between 5° and 7° C and (41°–45° F). Excellent results have been obtained with four species of rusts: *Cronartium filamentosum* (Peck) Hedgc., *C. stalactiforme* A. & K., *C. comandrae* Peck and *Peridermium harknessii* J. P. Moore. There seems no reason for anticipating that the method would not prove equally satisfactory for the storage of other *Cronartium* and *Peridermium* species. —JAMES L. MIELKE, Intermountain Forest and Range Expt. Sta., Logan, Utah.

VOLUME XLVI—CORRECTIONS

p. 58. Tiffany and Gilman: *Colletotrichum*. *C. lineola pachyspora* Ellis & Keller. North Am. Fungi *2183* instead of *2181*.

p. 632. Farrow: Tropical Soil Fungi. "Six major areas in the Panama Canal Zone and Costa Rica" should read "Three major areas in the Panama Canal Zone, two in the Province of Chiriquí and two in Costa Rica."

The regrettable delay in the appearance of the November-December issue of 1954 was due to loss of page proof in the mail.

[1] At the present time, the writer is unaware of a vote, favorable or unfavorable, in regard to his own renewed proposal to conserve *Rhodophyllus* Quél.

REVIEWS

LES DISCOMYCETES DE MADAGASCAR, by Marcelle Le Gal. (Prodrome à une Flore Mycologique de Madagascar et Dépendances, Vol. IV.) 465 pp., 172 figs., 1 map. Muséum National d'Histoire Naturelle, Paris. May 1953. Price 6500 fr. in France, 8000 fr. foreign (about $19.00 to $23.00).

Dr. Le Gal has treated in great detail 80 of the 85 species of discomycetes reported from Madagascar, but her discussions and conclusions have bearing on many European and North American genera and species. The volume is another significant contribution to our knowledge of this group prepared by one of its most able investigators. It can only be regretted that the high price of the book will prevent its wider dissemination and use.

Mme. Le Gal is gifted with the flair for taxonomic mycology that has been evident in all the great masters of mycology in France. Mycologists of other countries and other disciplines can rejoice in the fact that she has made an attempt here to bring French mycology in accord with mycology elsewhere by adhering to the International Code of Nomenclature. We may sincerely hope that her example will be followed by her countrymen, who hold her in high esteem. (She has recently been elected to the presidency of the Société Mycologique de France.)

Excellent descriptions and beautiful drawings make this one of the most useful floras yet to appear. Mme. Le Gal's meticulous attention to detail and her faithfulness to the type concept (including the necessity of examining type material when available) combine to stamp her taxonomic conclusions as authoritative. The great value of this work to discomycete systematists in other areas of the world, however, will lie in the critical comments, dispersed throughout the book, on extralimital species and genera. These reveal the breadth of Mme. Le Gal's understanding of the discomycetes.

Worthy of particular mention are the extended treatments of *Cookeina*, *Phillipsia* and *Sarcoscypha* (as *Plectania*), genera which hitherto presented many seemingly insolvable puzzles. Her unravelling of the misinformation and complex synonymy in the genus *Psilopezia* solves some of our knottiest taxonomic problems; the establishment of a single new genus, *Phaedropezia*, appears justified. For any student of the phylogeny of the discomycetes, Mme. Le Gal's proposed derivation of the Sarcoscyphaceae from *Rutstroemia* will provide stimulating food for thought and conjecture.

The bulk of this impressive work is devoted to descriptions and comments on the Madagascar species and genera, both operculate and inoperculate, and on their allies in other areas. A much shorter second part deals with the phytogeography and phylogeny of discomycetes. Latin diagnoses, an extensive bibliography, and good indices complete the volume.

Despite an honest effort by the author to follow the International Code of Nomenclature, the work displays a number of nomenclatural flaws. Three examples will suffice. (1) The genus *Phaeopezia* Sacc. is proposed (p. 80) for rejection on the grounds that it contains many diverse elements—but its holotype is well known! An originally monotypic genus can certainly not be discarded merely because of what is added later. (2) In uniting *Anthopeziza* Wettst. and *Microstoma* Berns., the former name is used (pp. 290, 291) in spite of the apparent priority of the latter. (3) *Sarcosoma* Casp. is used in a sense which excludes its holotype, in violation of the Code (pp. 201–206). In addition, some of the species and genera are cited in an ambiguous manner. New combinations, when made in the body of the text, are not precisely indicated by the use of the words "comb. nov.," by boldface type, or by indexing. The common *Peziza clypeata* of North America is transferred to *Pachyella*, but what page should a future author cite? The combination is first mentioned on page 27, but no basionym is mentioned there; the basionym and place of its publication do appear on page 179, but here the combination *Pachyella clypeata* is only inferred; on pages 414 and 415 the combination appears, but without author citation. A number of other cases of this sort could be mentioned in which it is not wholly certain whether the names are validly published under the Code. Presumably by an oversight, *Scutellinia colensoi* (Mass.) ex Le Gal nov. comb. [*sic*], which is actually a new species, is not provided with a validating Latin diagnosis.

The several nomenclatural errors should not, however, detract from the exceptionally important taxonomic contributions made in this work. In the opinion of this reviewer it ranks with Professor Nannfeldt's "Studien über die Morphologie . . . etc." as one of the two major works on Discomycete taxonomy since the appearance of Boudier's "Histoire et Classification . . . etc." in 1907.—RICHARD P. KORF, Dept. Plant Pathology, Cornell University.

DIE GATTUNGEN DER AMEROSPOREN PYRENOMYCETEN, by J. A. von Arx and Emil Müller. (Beiträge zur Kryptogamenflora der Schweiz, vol. 11, No. 1.) 434 pp., 119 figs. Büchler & Co., Berne, 1954. Price 3000 Swiss fcs. (about $8.00).

In this notable volume von Arx and Müller have begun the formidable and long-delayed task of revision and systematic organization of all genera of the Pyrenomycetes. Although the work of revision has been considerably advanced in the past half century, particularly by von Hoehnel and Petrak, the literature has been fragmentary and difficult to consult. This comprehensive treatment by von Arx and Müller makes the results of their revision conveniently available. It will prove indispensable to anyone with even an incidental interest in the Ascomycetes.

Considering the present confused state of classification in the Ascomycetes, the authors have wisely approached the problem through the comparison of all genera of similar ascospore form in the Saccardian system rather than the study of supposedly natural groups. In this first volume they treat the amerosporous genera of the Ascoloculares, true Pyrenomycetes, and Phacidiales, an order which they consider intermediate between the Ascoloculares and Discomycetes, but arbitrarily exclude the forms with allantoid ascospores and the well known genera of the Erysiphaceae. They present revised descriptions of 116 recognized genera with their numerous synonyms and append notes on 59 uncertain or excluded genera. Descriptions of most of the genera are accompanied by excellent drawings illustrating the characteristics of one or more typical species. Many of the genera, such as *Physalospora, Glomerella, Polystigma, Coccostroma,* etc., are monographed. For some genera only representative species are described. The treatment of genera and species generally is rather conservative. *Physalospora malorum* Shear, for example, appears in the list of more than 100 synonyms of *Botryosphaeria quercuum* (Schw.) Sacc., while *B. dothidea* (Moug. ex Fr.) Ces. & de Not. includes the ubiquitous *B. ribis* Gross & Dugg. among its 24 synonyms.

The value of the work is enhanced by the organization of the genera studied into families and orders. Keyes are provided to orders, families, and genera. In the Ascoloculares von Arx and Müller recognize the three orders Myriangiales, Pseudosphaeriales, and Dothiorales. All amerosporous genera are included in the families Botryosphaeriaceae (*Botryosphaeria, Guignardia, Vestergrenia, Trabutia,* etc.), Entopeltaceae, and Mesnieraceae of the Dothiorales. Genera are described in the families Cryptomycetaceae, Phacidiaceae, and Hypodermataceae of the Phacidiales. Amerosporous genera of the Sphaeriales are placed in the families Melanosporaceae, which includes the Chaetomiaceae; Polystigmataceae with genera such as *Glomerella, Polystigma, Phyllachora, Physalospora,* and *Coccostroma;* Cryptosporellaceae; Nectriaceae; and

Xylariaceae. The Xylariaceae includes most of the genera of the Sordariaceae such as *Sordaria, Gelasinospora,* and *Neurospora* as well as *Neocosmopara* and the genera usually associated with this family. The order Diaporthales is recognized for the allantoid Valsaceae and the Diaporthaceae, which contains amerosporous genera such as *Phomatospora, Endoxyla, Gnomoniella,* and *Diaporthopsis.* An outstanding feature is the account of the little known family Coronophoraceae. Von Arx and Müller provide the first clear description of this family and illustrate the structure of the ascocarp in this group with figures of representative species.

So many debatable points in the organization of families and treatment of individual genera are apparent even on superficial examination that it seems hardly worth while to cite examples of personal interest. This work will be severely criticized by specialists in various groups. Such criticism is to be expected, especially in a work of such magnitude, and should be welcomed. However, it is to be hoped that nothing will discourage the completion of this great undertaking. The work in itself represents an invaluable contribution to the development of a comprehensive classification of the Ascomycetes and will be of even greater importance as a basis for further intensive taxonomic study of restricted groups.—E. S. LUTTRELL.

PRACTICAL MYCOLOGY. MANUAL FOR IDENTIFICATION OF FUNGI, by Sigurd Funder. 146 pp., about 200 illustrations. Brøggers Boktr. Torlag, Oslo, Norway—Stechert-Hafner, Inc., New York. Price $6.50.

As stated in the introduction, this book is intended primarily for fractical workers who have little knowledge of the fungi but encounter them or the results of their activities in their work. A concise introductory chapter covers the major characteristics of the fungi and the types of fructifications they produce, together with simple laboratory procedures. This is followed by three sections on fungi of interest in general mycology, in medical mycology and in plant pathology, a classification in tabular form, references, glossary and index. The numerous illustrations are clear, rather diagrammatic and almost always helpful.

The treatment is very conservative: a desirable feature in a book intended for users who are not and usually do not care to be specialists. It should prove extremely useful in chemical and medical laboratories and has a place in mycology and plant pathology laboratories, particularly as a ready reference available to students.—G. W. M.

SCIENTIFIC RESEARCHES OF THE OZEGAHARA MOOR, by various specialists under the direction of Y. Ogura. xvi + 841 pp. 24 photo-

graphic plates with 52 photographs, 2 col. pl., numerous photographs and illustrations in text, separately numbered for each article. Tokyo, 1954.

The Ozegahara Moor has long been celebrated in Japan for its rich flora and insect life. Although a part of the Nikko National Park, it will be flooded if plans to construct a reservoir by damming the Padamigawa River are carried out. In view of this impending fate, it seemed desirable to record the vegetation and insect life while there was yet time. The handsome volume here noted is the result.

The text is in English and Japanese, usually with summaries in the alternative language. Of interest to mycologists are the following papers:

Lichen vegetation of Ozegahara moor and its vicinities, by Y. Asahina (537–538).

Wood-rotting fungous flora in the Ozegahara moor and its adjacent districts, by T. Sasaki (539–552).

Sphagnicolous fungi found in the Ozegahara moor, by Y. Kobayashi (553–560), including descriptions of three new species of agarics.

Studies on the aquatic fungi of the Ozegahara moor, by Y. Kobayashi and M. Okubo (561–574), including description of a new species of *Rhizidiomyces.*

Moulds found in the Ozegahara moor and its neighboring region, by Y. Kobayashi and K. Tsubaki (576–584).

In a separate folder are two large-scale vegetation maps and, on smaller scales, a map of the Ozegahara basin and a geologic map of the area.—G. W. M.

MANUSCRIPT

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